it is impossible to predict when the dissociation of the drug-protein complex is rate limiting from the association constants alone. It seems probable, however, that the rate of dissociation of the drug-protein complex seldom becomes rate limiting in the metabolism of drugs.

It seems more probable that diffusion of the free drug from the plasma into hepatocytes may be the rate-limiting step in the metabolism of drugs by highly active enzymes in the liver. When this occurs, the bound form of the drug in plasma remains in equilibrium with its free form as the concentration of unbound drug in plasma declines during the passage of the blood through the hepatic sinusoids. However, it is also possible that drug-binding proteins in the cytoplasm of hepatocytes hasten metabolism by maintaining the concentration gradient across the membranes of the hepatocytes and by increasing the amount of drug available to the enzymes located toward the central parts of the cells. Indeed, Y and Z proteins [18–21] are thought to act as carriers of sulfobromophthalein and other anions. But, whether they actually act as carriers or as tissue stores of the anions depends on a number of factors that include the association constants and the concentrations of the proteins as well as on the maximum rate and the K_m values of the drug-metabolizing enzymes and the relative diffusivities of the unbound drug and drug-protein complexes. Because of the complexities of these interrelationships, it is frequently difficult to determine when binding sites in hepatocytes serve as transport mechanisms or as storage mechanisms.

To determine whether plasma proteins act as transport carriers, it is necessary to calculate the apparent clearance on the basis of the concentration of unbound drug in plasma and to compare these apparent clearance values (rate of elimination/concentration of unbound drug) with hepatic blood flow rate. If the apparent clearance exceeds the hepatic blood flow when the drug is known to be eliminated solely by the liver, it may be concluded that the plasma proteins or other components in blood act as transport carriers. When it is not known whether a drug is eliminated solely by the liver, it would be useful to determine whether the clearance value exceeds the cardiac output, because the true clearance can never exceed the cardiac output.

With kind regards

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From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Friday, May 31, 2019 11:41 AM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry

<rgentry@ramboll.com>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>
Subject: RE: chloroprene -- Bayesian analysis

Harvey, all,
(Adding Dustin Kapraun: I'll catch you up later.)

Food for thought for June 12:

Regarding the in vitro analyses, the apparent discrepancy between the "Kg" experiments and the metabolic experiments leads to parameter uncertainty. Kg and the Km values can't be estimated independently from only the metabolic experiments, as you state in the manuscript. But to fully estimate the impact of that uncertainty in subsequent risk estimation, one could potentially use the results of a Bayesian analysis, not just the mean values, but the parameter distributions.

So the first estimation of Kgi and P (from data without microsomes) resulted in a joint distribution of these parameters. Some of those data are consistent with a higher P and Kgi than the mean values - the upper data in Figure B-1. Likewise your review of the literature on Km values effectively provides an informed prior on that parameter. Instead of fixing one or the other of these, formal Bayesian analysis could use those as priors when analyzing the data with active microsomes. I wonder if there are values of Kgi and P consistent with the upper end of the Kg-data (ie, within the uncertainty given those data) that are also consistent with the metabolic data?

I presume the Kg experiments, like the metabolic experiments, involved repeated measures, which needs to be properly accounted for in setting up the likelihood calculation in order to estimate the true uncertainty, full possible range of parameters. The number of independent experiments in Figure B-1 is a lot less than the number of data points, yes? (Since there are clusters of pink points at each time point, it's more than the number of colors used.) The estimated parameter uncertainty estimate would be too low if all the data are treated as independent. Likewise for the metabolic experiments.

If it wasn't done this way (accounting for repeated measures in the likelihood; formal Bayesian sequential parameter estimation), how hard would it be? To unpack this fully, and consider options, we may need to have original data by experimental unit (incubation vial), if it's not already set up that way.

-Paul

From: Schlosser, Paul
Sent: Thursday, May 30, 2019 8:06 AM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: chloroprene

Harvey, all,
(Copying EPA folk)

In the manuscript, you suggest that binding to the microsomal protein, which wasn't present in the Kg-measurement experiments, could have altered the partitioning between air and liquid phases, thereby resulting in the changed mass transfer. If true, this could be expiained in the model by changing the water:air partition coefficient. Maybe you can test the hypothesis that way ahead of our meeting, so we

know if it's a valid explanation or not. The microsomal concentration was 0.5 mg/ml, ~ 0.05%, so I don't know that it could alter partitioning too much, but it would be good to know ahead of the 12th if changing the PC alone to any extent could explain the apparent discrepancy.

-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Tuesday, May 14, 2019 4:40 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>
Subject: chloroprene

Hi Paul

Here is the revised manuscript on the chloroprene PBPK model, plus all of the supplemental materials that can be sent via email. The R model and two additional supplemental files (the IISRP report on the in vivo study and the Teklab report on the Kg study) will be transmitted separately, but I don't think you will really need to look at them at this point.

I'm going to be in Netherlands next week for Alina Efremenko's PhD ceremony, so it would be great if we could get together sometime this week to talk about the new analyses documented in the paper. Would that be possible? Jerry and I are free pretty much any time.

With kind regards
Harvey Clewell
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Message

Sent: 6/6/2019 11:58:07 AM
To: Harvey Clewell [HClewell@ramboll.com]
CC: Jerry Campbell [JCampbell@ramboll.com]; Michael Dzierlenga [MDZIERLENGA@ramboll.com]; Robinan Gentry [rgentry@ramboll.com]; r[Personal Matters / Ex. 6]n]; Thayer, Kris [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3ce4ae3f107749c6815f243260df98c3-Thayer, Kri]; Vandenberg, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=dcae2b98a04540fb8d099f9d4dead690-Vandenberg, John]; Bahadori, Tina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7da7967dcafb4c5bbc39c666fee31ec3-Bahadori, Tina]; Morozov, Viktor [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=03cc9abb639c453fabc2bbb3e4617228-Morozov, Viktor]; Davis, Allen [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=a8ecee8c29c54092b969e9547ea72596-Davis, Allen]; Sasso, Alan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8cb867519abc4dcea88149d12ef3e8e9-Sasso, Alan]; Kapraun, Dustin [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3a53c151b92a472fbfb295ed5df982a7-Kapraun, Du]; Sonja Sax [SSax@ramboll.com]; Ken Mundt [kenneth.mundt@cardno.com]; Miyoung Yoon [Personal Matters / Ex. 6]; Kenyon, Elaina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=0395d5b93f214c8ca49066f498f7d5c9-Kenyon, Elaina]
Subject: RE: chloroprene -- in vitro system

Harvey, all,

I saw Kris' and Tina's notes, but started this response last night, so it doesn't have those in the thread…

The question here is not the validity of the data, but the interpretation and application of those data. The metabolic data are not direct measurements of the rate of metabolism in the microsomal environment in which it occurs, but measurements of CP concentration in the headspace above an incubation mixture, which is 99.95% aqueous buffer. A computational model is used to estimate apparent Vmax and Km values in the mixture based on those data. The question is whether that computational model and hence the way in which the parameters are applied for use in a PBPK model are valid.

My understanding is that way back it was recognized that the apparent Km from such experiments could not be accurately applied to the *tissue* concentrations predicted by PBPK models: it doesn't match the in vivo data if you do that. The explanation was that the apparent Km obtained in vitro represents the average concentration in the incubation medium, not the concentration in the microsomes, at the enzyme active site, and that the appropriate corresponding concentration in the PBPK model is the venous blood concentration. That's why the "correct" form of PBPK models uses C_{venous} in the metabolism rate term, not C_{tissue}. Under these assumptions, the Km from an in vitro system such as this can be applied.

Underpinning this assumption is an assumption of rapid equilibrium between the aqueous

First, the blank experiments where Matt measured the system losses in the absence of metabolism show that there is at best minimal non-specific binding, with which the microsomes might compete. When measuring partition coefficients, tissues are homogenized in buffer and it is assumed that the volume of the buffer and the water:air PC can be used to calculate how much of the gas is in the buffer, by mass balance how much is in the tissue. If I assume that the microsome:air PC is the same as liver:air, by the same type of calculation, the extent to which that could impact partitioning from the air to the incubation medium is negligible, since the microsomes are only 0.05% of the medium. To argue with that is to argue with the method by which PCs are estimated.

The data are valid, I am not arguing that. But the data are measurements of gas concentration in the headspace above an incubation mixture, *not* direct measurements of the rate of reaction as a function of concentration in the incubation medium. These data are being interpreted, to extract metabolic parameters, using a model that assumes the system is well-mixed and that the metabolic parameters can be estimated based on the amount removed from the headspace, and the water:air partition coefficient; i.e., assuming the same concentration in the entire mixture.

If it were somehow true that the microsomes significantly altered the partitioning, then this computational estimate of concentration in the microsomes where the enzyme is functioning is also wrong, hence the estimated kinetic parameters would be wrong.

I am working under the assumption that Denka paid to collect are also valid data, and are applicable to the in vitro metabolism experiments.

And I don't think we need to make rather stretched hypotheses about what a small amount of microsomes might do to partitioning to explain the discrepancy. But what I do believe that the combined data show is that the computational model being used to interpret the data is incorrect: the data are valid, but there's a problem with the model. In particular, I don't believe you can have the resistance to mass transfer that was measured and complete mixing in a system where metabolism is that high. It only appears to be a discrepancy when you assume the system is well mixed.

I've set up an alternate model where the liquid phase is divided into 10 layers. I just have very preliminary results, need to do some more testing, but I believe it can fit both the Kg data and the metabolic data with a common set of parameters.

On the one hand, you are right that we don't know the exact mixing speed that Matt used, etc. On the other hand, you obtained a Kg which is remarkably, remarkably close to the one I estimated for benzene way back. I don't think that's just a coincidence, but is saying that the resistance to diffusion in water is similar for small VOCs whose molecular weights are not too far apart.

I did suggest trying to fit the Kg to the data, but I didn't think you would get something that much higher than was measured. I don't believe that 0.05% MSP can cause a 20-fold increase in the rate of mass transfer, no way.

When we talked and Jerry showed me the results a few weeks back, I couldn't think of a better explanation. But still, the discrepancy was there… Who is it that said models are most useful when they fail, because that teaches us something? To argue away part of the data just because it makes the model fail is not learning from the failure. I'm sorry it's taken me so long to have this realization about mixing, but it may matter for the interpretation of other data sets where the in vivo metabolism is not rate limiting. And it may matter in estimating the lung parameters, which are key to that metric.

I'm pretty sure that I also suggested that for the data sets which could be fitted with the Kg as measured, you use that Kg, not the one fitted to the liver data. That's not what was done, and I'll want to see if it makes any difference for the lung metabolism simulations.

By the way, in the script I have for the mouse liver metabolism the protein content was set to be 1 mg/ml, but Matt's paper says it was 0.5 mg/ml. So that would impact the IVIVE by a factor of 2, if it wasn't caught/corrected in the latest revision.

It is a shame if no one is collecting such data now, since that would mean using default methods for VOCs where there aren't the data. I thought we still have labs and equipment here where they can be done. Whether it's important enough to do for this particular chemical, or to further our ability to interpret such data for other VOCs is a question. The plots that John Wambaugh shows comparing IVIVE predictions to actual PK data indicate it can be off by an order of magnitude or more. So for chemicals where there are no in vivo data we either build that uncertainty into the risk estimation, or we find a way to reduce it. Improving our ability to interpret in vitro data with a limited set of experiments, even if they can't be done by a contract lab, automated methods, might be worthwhile if it can reduce the prediction error across multiple chemicals.

In the end I believe that if a model is based on assumptions that are invalidated by the data in hand, then the parameters that come from using that model are uncertain, even if the data are valid. In this case the uncertainty may be acceptable, because the in vivo PK is flow limited. But I want to complete a bit more analysis with the multi-layer model before convincing myself of that.

-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Wednesday, June 05, 2019 6:34 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen <Personal Matters / Ex. 6 hayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon <Personal Matters / Ex. 6 Kenyon, Elaina <Kenyon.Elaina@epa.gov>
Subject: RE: chloroprene -- in vitro system

Hi Paul
, to investigate your hypothesis regarding diffusion limitation in the liquid phase. However, even though the experiment you describe may sound simple, performing it correctly would be just as difficult as the original studies conducted by

Matt Himmelstein. Unfortunately, there is no longer anyone conducting these kinds of studies. Both John Wambaugh at NCCT and I have tried to identify laboratories with experience in conducting in vitro metabolism studies with volatiles, but we have both been unsuccessful. That is why Denka had to use an environmental contract laboratory to conduct the Kg study.

I have discussed this question with Miyoung Yoon, who is now at FDA, and it was she who suggested that the presence of microsomes in Matt's studies would have greatly increased the availability of chloroprene for metabolism by competing with other sources of non-specific binding. She is the most experienced researcher in the area of *in vitro* metabolism that I know of. I'm afraid this difference in opinion will need to go unresolved, however, not only because the necessary studies are impractical but also because the relevance of any new results to Matt's published studies would be highly uncertain. The difficulty is that Kg is just an empirical parameter that represents the rate of mass transfer under specific experimental conditions. Most importantly, as mixing is increased, the transition from simple diffusion to laminar convection and then to turbulent convection impacts the rate of mass transfer in a nonlinear manner, so extrapolation from one experiment to another would be extremely difficult.

I have also discussed this question with Mel Andersen, and he believes that the published *in vitro* data are completely reliable. He agrees with the approach you suggested for estimating Kg from the male mouse liver metabolism data by fixing Km at the value of 1 uM supported by the literature on cyp2E1 substrates. Re-estimating the metabolism parameters with the estimated Kg results in a 25% decrease in risk compared to using the published values. Values of Kg lower than the value estimated from the metabolism data would reduce risk estimates even further. I just don't see the benefit of performing any additional studies.

With kind regards
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From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Wednesday, June 5, 2019 9:57 AM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen <Personal Matters / Ex. 6>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon <Personal Matters / Ex. 6>; Kenyon, Elaina <Kenyon.Elaina@epa.gov>
Subject: RE: chloroprene -- in vitro system

I've changed the subject to better reflect the topic.

This morning I realized there's actually a fairly simple experiment that could determine if my hypothesis on diffusion limitation in the liquid phase is correct: run incubations with ½ (or less) of the total incubation mixture, concentrations of microsomes, etc, otherwise the same. (We'd want to have parallel experiments, same lab, same microsomes, etc., with full volume.)

If the system is well mixed, as the current model suggests, then the rate at which chloroprene is removed from the headspace (mass/time) would be reduced by ½, since there's ½ the microsomes doing the work. You'd have to incorporate the change in head-space volume in the calculation (either way).

Alternately, if there is diffusion limitation, with the microsomes near the surface doing the bulk of the metabolism, then the rate of chloroprene removal would not be reduced by ½. A higher fraction of the microsomes would be near the air:liquid interface, so the rate of removal per mg microsome in the system would be higher.

Since the concentration in the incubation solution is effectively calculated by mass balance, this would also lead to an increase in the estimated concentration associated with a given rate of metabolism, I'm pretty sure. The result would then also be an increase in the apparent Km.

Doing these experiments would then evaluate the extent to which mass transport in the liquid phase is limiting in this system, using live/active microsomes, doesn't require any more elaborate analytic methods than already employed.

-Paul

From: Schlosser, Paul
Sent: Tuesday, June 04, 2019 5:14 PM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen <Personal Matters / Ex. 6>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon
Personal Matters / Ex. 6
Subject: RE: chloroprene -- Bayesian analysis

Harvey, all,

In the Kg experiments, if the sampling of the liquid phase is well into the liquid, away from the air:liquid interface, but mixing is sufficient to keep microsomes evenly distributed, then it's possible for the CP concentration near the surface to be higher, less limited by mass transfer resistance, than in the middle or bottom. If the microsomes near the surface are responsible for the majority of the metabolism, then that could explain the discrepancy between the Kg data/model and the metabolic data.

But that would also mean that the activity of those microsomes was higher than currently estimated… if only 10% of the microsomes (those near the surface) are responsible for the metabolism, the actual Vmax would be 10x higher per mg microsomes, for example. Since the in vivo PK are flow limited, the fits to those data would be the same, if Vmax (in the liver) is actually 10x higher, those in vivo data don't invalidate this hypothesis.

The incubation results would still be linear with microsome content by this explanation, presuming they are well mixed. Using ½ the total microsomes would put that much less near the surface, resulting in a proportional decrease in removal from the gas phase. It's saying that under conditions of high metabolic activity, the assumption of a well-mixed incubation volume is not valid. I think that's more likely than a small fraction of microsomes significantly affecting transport through the entire volume.

Good evening, until tomorrow!

-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Tuesday, June 04, 2019 2:12 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen <Personal Matters / Ex. 6>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon
Personal Matters / Ex. 6
Subject: RE: chloroprene -- Bayesian analysis

Hi Paul

I do not agree that the apparent discrepancy between the Kg experiments and the metabolism experiments leads to parameter uncertainty. To break the collinearity between Km and Kg, we have followed your suggestion of fixing Km at a value based on the literature for cyp2e1 substrates (1 uM), and have re-estimated Vmax and Kg in the male mouse liver, which shows the highest rates of metabolism. The resulting value of Kg represents the maximum limitation on transport in the in vitro studies that is consistent with the data. It does not demonstrate, however, that there was any significant transport limitation in those studies.

Personally, I have complete confidence in the metabolism data collected by Matt Himmelstein and in the approach he used for its analysis. I see no evidence to support an assumption of transport limitation in his studies. The constants derived in the published analysis are consistent with a large body of work on *in vivo* estimation of kinetic constants for clearance of well-metabolized vapors (i.e., with relatively low Km values). Moreover, assuming that there was a significant limitation on transport in these studies results in Km values that are implausibly low, which in turn results in lower risk estimate compared to use of the published values.

The discussion below of the role of plasma proteins on metabolism is from James Gillette's 1973 paper in the Annals of the NY Academy of Science. He suggested that binding proteins in the plasma can accelerate metabolism by acting as carriers of a drug to the vicinity of the hepatocytes. I believe that microsomal proteins can play a similar role in the *in vitro* studies: in the presence of mixing, non-specific binding of a lipophilic compound to proteins can serve to overcome the transport limitation associated with penetrating the aqueous media. In other words, since chloroprene is lipophilic, diffusion through the aqueous media in the *in vitro* assay would normally be rate-limiting, but if the media is well-mixed and contains microsomal proteins, then non-specific binding of chloroprene to the microsomes could greatly enhance its availability for metabolism.

Unfortunately, investigating this effect in the *in vitro* system would not be at all straightforward, because the microsomal proteins serve both as the site of metabolism and as a source of non-specific binding that competes with the surface of the vial. In fact, I do not believe it is possible to experimentally determine a Kg that would be appropriate in any microsomal metabolism study, because of the dual role that the microsomes play (metabolism and non-specific binding). Denaturing the microsomal proteins in order to eliminate metabolism would also alter their tertiary binding structure.

it is impossible to predict when the dissociation of the drug-protein complex is rate limiting from the association constants alone. It seems probable, however, that the rate of dissociation of the drug-protein complex seldom becomes rate limiting in the metabolism of drugs.

It seems more probable that diffusion of the free drug from the plasma into hepatocytes may be the rate-limiting step in the metabolism of drugs by highly active enzymes in the liver. When this occurs, the bound form of the drug in plasma remains in equilibrium with its free form as the concentration of unbound drug in plasma declines during the passage of the blood through the hepatic sinusoids. However, it is also possible that drug-binding proteins in the cytoplasm of hepatocytes hasten metabolism by maintaining the concentration gradient across the membranes of the hepatocytes and by increasing the amount of drug available to the enzymes located toward the central parts of the cells. Indeed, Y and Z proteins [18–21] are thought to act as carriers of sulfobromophthalein and other anions. But, whether they actually act as carriers or as tissue stores of the anions depends on a number of factors that include the association constants and the concentrations of the proteins as well as on the maximum rate and the K_m values of the drug-metabolizing enzymes and the relative diffusivities of the unbound drug and drug-protein complexes. Because of the complexities of these interrelationships, it is frequently difficult to determine when binding sites in hepatocytes serve as transport mechanisms or as storage mechanisms.

To determine whether plasma proteins act as transport carriers, it is necessary to calculate the apparent clearance on the basis of the concentration of unbound drug in plasma and to compare these apparent clearance values (rate of elimination/concentration of unbound drug) with hepatic blood flow rate. If the apparent clearance exceeds the hepatic blood flow when the drug is known to be eliminated solely by the liver, it may be concluded that the plasma proteins or other components in blood act as transport carriers. When it is not known whether a drug is eliminated solely by the liver, it would be useful to determine whether the clearance value exceeds the cardiac output, because the true clearance can never exceed the cardiac output.

With kind regards
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From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Friday, May 31, 2019 11:41 AM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry

<rgentry@ramboll.com>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>
Subject: RE: chloroprene -- Bayesian analysis

Harvey, all,
(Adding Dustin Kapraun: I'll catch you up later.)

Food for thought for June 12:

Regarding the in vitro analyses, the apparent discrepancy between the "Kg" experiments and the metabolic experiments leads to parameter uncertainty. Kg and the Km values can't be estimated independently from only the metabolic experiments, as you state in the manuscript. But to fully estimate the impact of that uncertainty in subsequent risk estimation, one could potentially use the results of a Bayesian analysis, not just the mean values, but the parameter distributions.

So the first estimation of Kgi and P (from data without microsomes) resulted in a joint distribution of these parameters. Some of those data are consistent with a higher P and Kgi than the mean values – the upper data in Figure B-1. Likewise your review of the literature on Km values effectively provides an informed prior on that parameter. Instead of fixing one or the other of these, formal Bayesian analysis could use those as priors when analyzing the data with active microsomes. I wonder if there are values of Kgi and P consistent with the upper end of the Kg-data (ie, within the uncertainty given those data) that are also consistent with the metabolic data?

I presume the Kg experiments, like the metabolic experiments, involved repeated measures, which needs to be properly accounted for in setting up the likelihood calculation in order to estimate the true uncertainty, full possible range of parameters. The number of independent experiments in Figure B-1 is a lot less than the number of data points, yes? (Since there are clusters of pink points at each time point, it's more than the number of colors used.) The estimated parameter uncertainty estimate would be too low if all the data are treated as independent. Likewise for the metabolic experiments.

If it wasn't done this way (accounting for repeated measures in the likelihood; formal Bayesian sequential parameter estimation), how hard would it be? To unpack this fully, and consider options, we may need to have original data by experimental unit (incubation vial), if it's not already set up that way.

-Paul

From: Schlosser, Paul
Sent: Thursday, May 30, 2019 8:06 AM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: chloroprene

Harvey, all,
(Copying EPA folk)

In the manuscript, you suggest that binding to the microsomal protein, which wasn't present in the Kg-measurement experiments, could have altered the partitioning between air and liquid phases, thereby resulting in the changed mass transfer. If true, this could be explained in the model by changing the water:air partition coefficient. Maybe you can test the hypothesis that way ahead of our meeting, so we know if it's a valid explanation or not. The microsomal concentration was 0.5 mg/ml, ~0.05%, so I don't know that it could alter partitioning too much, but it would be good to know ahead of the 12th if changing the PC alone to any extent could explain the apparent discrepancy.

-Paul

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Sent: Tuesday, May 14, 2019 4:40 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>
Subject: chloroprene

Hi Paul

Here is the revised manuscript on the chloroprene PBPK model, plus all of the supplemental materials that can be sent via email. The R model and two additional supplemental files (the IISRP report on the in vivo study and the Teklab report on the Kg study) will be transmitted separately, but I don't think you will really need to look at them at this point.

I'm going to be in Netherlands next week for Alina Efremenko's PhD ceremony, so it would be great if we could get together sometime this week to talk about the new analyses documented in the paper. Would that be possible? Jerry and I are free pretty much any time.

With kind regards
Harvey Clewell
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Message

Sent: 6/6/2019 11:52:30 AM
To: Harvey Clewell [HClewell@ramboll.com]
CC: Jerry Campbell [JCampbell@ramboll.com]; Michael Dzierlenga [MDZIERLENGA@ramboll.com]; Robinan Gentry [rgentry@ramboll.com]; manderse[Personal Matters / Ex. 6] Thayer, Kris [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3ce4ae3f107749c6815f243260df98c3-Thayer, Kri]; Vandenberg, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=dcae2b98a04540fb8d099f9d4dead690-Vandenberg, John]; Bahadori, Tina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7da7967dcafb4c5bbc39c666fee31ec3-Bahadori, Tina]; Morozov, Viktor [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=03cc9abb639c453fabc2bbb3e4617228-Morozov, Viktor]; Davis, Allen [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=a8ecee8c29c54092b969e9547ea72596-Davis, Allen]; Sasso, Alan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8cb867519abc4dcea88149d12ef3e8e9-Sasso, Alan]; Kapraun, Dustin [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3a53c151b92a472fbfb295ed5df982a7-Kapraun, Du]; Sonja Sax [SSax@ramboll.com]; Ken Mundt [kenneth.mundt@cardno.com]; Miyoung Yoon [Personal Matters / Ex. 6] Kenyon, Elaina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=0395d5b93f214c8ca49066f498f7d5c9-Kenyon, Elaina]
Subject: RE: chloroprene -- in vitro system

Harvey, all,

I saw Kris' and Tina's notes, but started this response last night, so it doesn't have those in the thread…

The question here is not the validity of the data, but the interpretation and application of those data. The metabolic data are not direct measurements of the rate of metabolism in the microsomal environment in which it occurs, but measurements of CP concentration in the headspace above an incubation mixture, which is 99.95% aqueous buffer. A computational model is used to estimate apparent Vmax and Km values in the mixture based on those data. The question is whether that computational model and hence the way in which the parameters are applied for use in a PBPK model are valid.

My understanding is that way back it was recognized

First, the blank experiments where Matt measured the system losses in the absence of metabolism show that there is at best minimal non-specific binding, with which the microsomes might compete. When measuring partition coefficients, tissues are homogenized in buffer and it is assumed that the volume of the buffer and the water:air PC can be used to calculate how much of the gas is in the buffer, by mass balance how much is in the tissue. If I assume that the microsome:air PC is the same as liver:air, by the same type of calculation, the extent to which that could impact partitioning from the air to the incubation medium is negligible, since the microsomes are only 0.05% of the medium. To argue with that is to argue with the method by which PCs are estimated.

The data are valid, I am not arguing that. But the data are measurements of gas concentration in the headspace above an incubation mixture, ***not*** direct measurements of the rate of reaction as a function of concentration in the incubation medium. These data are being interpreted, to extract metabolic parameters, using a model that assumes the system is well-mixed and that the metabolic parameters can be estimated based on the amount removed from the headspace, and the water:air partition coefficient; i.e., assuming the same concentration in the entire mixture.

If it were somehow true that the microsomes significantly altered the partitioning, then this computational estimate of concentration in the microsomes where the enzyme is functioning is also wrong, hence the estimated kinetic parameters would be wrong.

I am working under the assumption that Denka paid to collect are also valid data, and are applicable to the in vitro metabolism experiments.

And I don't think we need to make rather stretched hypotheses about what a small amount of microsomes might do to partitioning to explain the discrepancy. But what I do believe that the combined data show is that the computational model being used to interpret the data is incorrect: the data are valid, but there's a problem with the model. In particular, I don't believe you can have the resistance

to mass transfer that was measured and complete mixing in a system where metabolism is that high. It only appears to be a discrepancy when you assume the system is well mixed.

I've set up an alternate model where the liquid phase is divided into 10 layers. I just have very preliminary results, need to do some more testing, but I believe it can fit both the Kg data and the metabolic data with a common set of parameters.

On the one hand, you are right that we don't know the exact mixing speed that Matt used, etc. On the other hand, you obtained a Kg which is remarkably, remarkably close to the one I estimated for benzene way back. I don't think that's just a coincidence, but is saying that the resistance to diffusion in water is similar for small VOCs whose molecular weights are not too far apart.

I did suggest trying to fit the Kg to the data, but I didn't think you would get something that much higher than was measured. I don't believe that 0.05% MSP can cause a 20-fold increase in the rate of mass transfer, no way.

When we talked and Jerry showed me the results a few weeks back, I couldn't think of a better explanation. But still, the discrepancy was there… Who is it that said models are most useful when they fail, because that teaches us something? To argue away part of the data just because it makes the model fail is not learning from the failure. I'm sorry it's taken me so long to have this realization about mixing, but it may matter for the interpretation of other data sets where the in vivo metabolism is not rate limiting. And it may matter in estimating the lung parameters, which are key to that metric.

I'm pretty sure that I also suggested that for the data sets which could be fitted with the Kg as measured, you use that Kg, not the one fitted to the liver data. That's not what was done, and I'll want to see if it makes any difference for the lung metabolism simulations.

By the way, in the script I have for the mouse liver metabolism the protein content was set to be 1 mg/ml, but Matt's paper says it was 0.5 mg/ml. So that would impact the IVIVE by a factor of 2, if it wasn't caught/corrected in the latest revision.

It is a shame if no one is collecting such data now, since that would mean using default methods for VOCs where there aren't the data. I thought we still have labs and equipment here where they can be done. Whether it's important enough to do for this particular chemical, or to further our ability to interpret such data for other VOCs is a question. The plots that John Wambaugh shows comparing IVIVE predictions to actual PK data indicate it can be off by an order of magnitude or more. So for chemicals where there are no in vivo data we either build that uncertainty into the risk estimation, or we find a way to reduce it. Improving our ability to interpret in vitro data with a limited set of experiments, even if they can't be done by a contract lab, automated methods, might be worthwhile if it can reduce the prediction error across multiple chemicals.

In the end I believe that if a model is based on assumptions that are invalidated by the data in hand, then the parameters that come from using that model are uncertain, even if the data are valid. In this case the uncertainty may be acceptable, because the in vivo PK is flow limited. But I want to complete a bit more analysis with the multi-layer model before convincing myself of that.

-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Wednesday, June 05, 2019 6:34 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen Personal Matters / Ex. 6 Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon Personal Matters / Ex. 6 >; Kenyon, Elaina <Kenyon.Elaina@epa.gov>
Subject: RE: chloroprene -- in vitro system

Hi Paul
, to investigate your hypothesis regarding diffusion limitation in the liquid phase. However, even though the experiment you describe may sound simple, performing it correctly would be just as difficult as the original studies conducted by Matt Himmelstein. Unfortunately, there is no longer anyone conducting these kinds of studies. Both John Wambaugh at NCCT and I have tried to identify laboratories with experience in conducting in vitro metabolism studies with volatiles, but we have both been unsuccessful. That is why Denka had to use an environmental contract laboratory to conduct the Kg study.

I have discussed this question with Miyoung Yoon, who is now at FDA, and it was she who suggested that the presence of microsomes in Matt's studies would have greatly increased the availability of chloroprene for metabolism by competing with other sources of non-specific binding. She is the most experienced researcher in the area of *in vitro* metabolism that I know of. I'm afraid this difference in opinion will need to go unresolved, however, not only because the necessary studies are impractical but also because the relevance of any new results to Matt's published studies would be highly uncertain. The difficulty is that Kg is just an empirical parameter that represents the rate of mass transfer under specific experimental conditions. Most importantly, as mixing is increased, the transition from simple diffusion to laminar convection and then to turbulent convection impacts the rate of mass transfer in a nonlinear manner, so extrapolation from one experiment to another would be extremely difficult.

I have also discussed this question with Mel Andersen, and he believes that the published *in vitro* data are completely reliable. He agrees with the approach you suggested for estimating Kg from the male mouse liver metabolism data by fixing Km at the value of 1 uM supported by the literature on cyp2E1 substrates. Re-estimating the metabolism parameters with the estimated Kg results in a 25% decrease in risk compared to using the published values. Values of Kg lower than the value estimated from the metabolism data would reduce risk estimates even further. I just don't see the benefit of performing any additional studies.

With kind regards
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From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Wednesday, June 5, 2019 9:57 AM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen <Personal Matters / Ex. 6>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon <Personal Matters / Ex. 6>; Kenyon, Elaina <Kenyon.Elaina@epa.gov>
Subject: RE: chloroprene -- in vitro system

I've changed the subject to better reflect the topic.

This morning I realized there's actually a fairly simple experiment that could determine if my hypothesis on diffusion limitation in the liquid phase is correct: run incubations with ½ (or less) of the total incubation mixture, concentrations of microsomes, etc, otherwise the same. (We'd want to have parallel experiments, same lab, same microsomes, etc., with full volume.)

If the system is well mixed, as the current model suggests, then the rate at which chloroprene is removed from the headspace (mass/time) would be reduced by ½, since there's ½ the microsomes doing the work. You'd have to incorporate the change in head-space volume in the calculation (either way).

Alternately, if there is diffusion limitation, with the microsomes near the surface doing the bulk of the metabolism, then the rate of chloroprene removal would not be reduced by ½. A higher fraction of the microsomes would be near the air:liquid interface, so the rate of removal per mg microsome in the system would be higher.

Since the concentration in the incubation solution is effectively calculated by mass balance, this would also lead to an increase in the estimated concentration associated with a given rate of metabolism, I'm pretty sure. The result would then also be an increase in the apparent Km.

Doing these experiments would then evaluate the extent to which mass transport in the liquid phase is limiting in this system, using live/active microsomes, doesn't require any more elaborate analytic methods than already employed.

-Paul

From: Schlosser, Paul
Sent: Tuesday, June 04, 2019 5:14 PM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen <Personal Matters / Ex. 6>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon <Personal Matters / Ex. 6>
Subject: RE: chloroprene -- Bayesian analysis

Harvey, all,

In the Kg experiments, if the sampling of the liquid phase is well into the liquid, away from the air:liquid interface, but mixing is sufficient to keep microsomes evenly distributed, then it's possible for the CP concentration near the surface to be higher, less limited by mass transfer resistance, than in the middle or bottom. If the microsomes near the surface are responsible for the majority of the metabolism, then that could explain the discrepancy between the Kg data/model and the metabolic data.

But that would also mean that the activity of those microsomes was higher than currently estimated… if only 10% of the microsomes (those near the surface) are responsible for the metabolism, the actual Vmax would be 10x higher per mg microsomes, for example. Since the in vivo PK are flow limited, the fits to those data would be the same, if Vmax (in the liver) is actually 10x higher, those in vivo data don't invalidate this hypothesis.

The incubation results would still be linear with microsome content by this explanation, presuming they are well mixed. Using ½ the total microsomes would put that much less near the surface, resulting in a proportional decrease in removal from the gas phase. It's saying that under conditions of high metabolic activity, the assumption of a well-mixed incubation volume is not valid. I think that's more likely than a small fraction of microsomes significantly affecting transport through the entire volume.

Good evening, until tomorrow!

-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Tuesday, June 04, 2019 2:12 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen <Personal Matters / Ex. 6>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon <Personal Matters / Ex. 6>
Subject: RE: chloroprene -- Bayesian analysis

Hi Paul

I do not agree that the apparent discrepancy between the Kg experiments and the metabolism experiments leads to parameter uncertainty. To break the collinearity between Km and Kg, we have followed your suggestion of fixing Km at a value based on the literature for cyp2e1 substrates (1 uM), and have re-estimated Vmax and Kg in the male mouse liver, which shows the highest rates of metabolism. The resulting value of Kg represents the maximum limitation on

transport in the in vitro studies that is consistent with the data. It does not demonstrate, however, that there was any significant transport limitation in those studies.

Personally, I have complete confidence in the metabolism data collected by Matt Himmelstein and in the approach he used for its analysis. I see no evidence to support an assumption of transport limitation in his studies. The constants derived in the published analysis are consistent with a large body of work on *in vivo* estimation of kinetic constants for clearance of well-metabolized vapors (i.e., with relatively low Km values). Moreover, assuming that there was a significant limitation on transport in these studies results in Km values that are implausibly low, which in turn results in lower risk estimate compared to use of the published values.

The discussion below of the role of plasma proteins on metabolism is from James Gillette's 1973 paper in the Annals of the NY Academy of Science. He suggested that binding proteins in the plasma can accelerate metabolism by acting as carriers of a drug to the vicinity of the hepatocytes. I believe that microsomal proteins can play a similar role in the *in vitro* studies: in the presence of mixing, non-specific binding of a lipophilic compound to proteins can serve to overcome the transport limitation associated with penetrating the aqueous media. In other words, since chloroprene is lipophilic, diffusion through the aqueous media in the *in vitro* assay would normally be rate-limiting, but if the media is well-mixed and contains microsomal proteins, then non-specific binding of chloroprene to the microsomes could greatly enhance its availability for metabolism.

Unfortunately, investigating this effect in the *in vitro* system would not be at all straightforward, because the microsomal proteins serve both as the site of metabolism and as a source of non-specific binding that competes with the surface of the vial. In fact, I do not believe it is possible to experimentally determine a Kg that would be appropriate in any microsomal metabolism study, because of the dual role that the microsomes play (metabolism and non-specific binding). Denaturing the microsomal proteins in order to eliminate metabolism would also alter their tertiary binding structure.

it is impossible to predict when the dissociation of the drug-protein complex is rate limiting from the association constants alone. It seems probable, however, that the rate of dissociation of the drug-protein complex seldom becomes rate limiting in the metabolism of drugs.

It seems more probable that diffusion of the free drug from the plasma into hepatocytes may be the rate-limiting step in the metabolism of drugs by highly active enzymes in the liver. When this occurs, the bound form of the drug in plasma remains in equilibrium with its free form as the concentration of unbound drug in plasma declines during the passage of the blood through the hepatic sinusoids. However, it is also possible that drug-binding proteins in the cytoplasm of hepatocytes hasten metabolism by maintaining the concentration gradient across the membranes of the hepatocytes and by increasing the amount of drug available to the enzymes located toward the central parts of the cells. Indeed, Y and Z proteins [18–21] are thought to act as carriers of sulfobromophthalein and other anions. But, whether they actually act as carriers or as tissue stores of the anions depends on a number of factors that include the association constants and the concentrations of the proteins as well as on the maximum rate and the K_m values of the drug-metabolizing enzymes and the relative diffusivities of the unbound drug and drug-protein complexes. Because of the complexities of these interrelationships, it is frequently difficult to determine when binding sites in hepatocytes serve as transport mechanisms or as storage mechanisms.

To determine whether plasma proteins act as transport carriers, it is necessary to calculate the apparent clearance on the basis of the concentration of unbound drug in plasma and to compare these apparent clearance values (rate of elimination/concentration of unbound drug) with hepatic blood flow rate. If the apparent clearance exceeds the hepatic blood flow when the drug is known to be eliminated solely by the liver, it may be concluded that the plasma proteins or other components in blood act as transport carriers. When it is not known whether a drug is eliminated solely by the liver, it would be useful to determine whether the clearance value exceeds the cardiac output, because the true clearance can never exceed the cardiac output.

With kind regards

Harvey Clewell
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Sent: Friday, May 31, 2019 11:41 AM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry

<rgentry@ramboll.com>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>
Subject: RE: chloroprene -- Bayesian analysis

Harvey, all,
(Adding Dustin Kapraun: I'll catch you up later.)

Food for thought for June 12:

Regarding the in vitro analyses, the apparent discrepancy between the "Kg" experiments and the metabolic experiments leads to parameter uncertainty. Kg and the Km values can't be estimated independently from only the metabolic experiments, as you state in the manuscript. But to fully estimate the impact of that uncertainty in subsequent risk estimation, one could potentially use the results of a Bayesian analysis, not just the mean values, but the parameter distributions.

So the first estimation of Kgi and P (from data without microsomes) resulted in a joint distribution of these parameters. Some of those data are consistent with a higher P and Kgi than the mean values – the upper data in Figure B-1. Likewise your review of the literature on Km values effectively provides an informed prior on that parameter. Instead of fixing one or the other of these, formal Bayesian analysis could use those as priors when analyzing the data with active microsomes. I wonder if there are values of Kgi and P consistent with the upper end of the Kg-data (ie, within the uncertainty given those data) that are also consistent with the metabolic data?

I presume the Kg experiments, like the metabolic experiments, involved repeated measures, which needs to be properly accounted for in setting up the likelihood calculation in order to estimate the true uncertainty, full possible range of parameters. The number of independent experiments in Figure B-1 is a lot less than the number of data points, yes? (Since there are clusters of pink points at each time point, it's more than the number of colors used.) The estimated parameter uncertainty estimate would be too low if all the data are treated as independent. Likewise for the metabolic experiments.

If it wasn't done this way (accounting for repeated measures in the likelihood; formal Bayesian sequential parameter estimation), how hard would it be? To unpack this fully, and consider options, we may need to have original data by experimental unit (incubation vial), if it's not already set up that way.

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Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: chloroprene

Harvey, all,
(Copying EPA folk)

In the manuscript, you suggest that binding to the microsomal protein, which wasn't present in the Kg-measurement experiments, could have altered the partitioning between air and liquid phases, thereby resulting in the changed mass transfer. If true, this could be explained in the model by changing the water:air partition coefficient. Maybe you can test the hypothesis that way ahead of our meeting, so we know if it's a valid explanation or not. The microsomal concentration was 0.5 mg/ml, ~0.05%, so I don't know that it could alter partitioning too much, but it would be good to know ahead of the 12th if changing the PC alone to any extent could explain the apparent discrepancy.

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Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>
Subject: chloroprene

Hi Paul

Here is the revised manuscript on the chloroprene PBPK model, plus all of the supplemental materials that can be sent via email. The R model and two additional supplemental files (the IISRP report on the in vivo study and the Teklab report on the Kg study) will be transmitted separately, but I don't think you will really need to look at them at this point.

I'm going to be in Netherlands next week for Alina Efremenko's PhD ceremony, so it would be great if we could get together sometime this week to talk about the new analyses documented in the paper. Would that be possible? Jerry and I are free pretty much any time.

With kind regards
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Message

Sent: 6/6/2019 11:47:03 AM
To: Harvey Clewell [HClewell@ramboll.com]
CC: Jerry Campbell [JCampbell@ramboll.com]; Michael Dzierlenga [MDZIERLENGA@ramboll.com]; Robinan Gentry [rgentry@ramboll.com]; mandersen [Personal Matters / Ex. 6]; Thayer, Kris [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3ce4ae3f107749c6815f243260df98c3-Thayer, Kri]; Vandenberg, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=dcae2b98a04540fb8d099f9d4dead690-Vandenberg, John]; Bahadori, Tina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7da7967dcafb4c5bbc39c666fee31ec3-Bahadori, Tina]; Morozov, Viktor [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=03cc9abb639c453fabc2bbb3e4617228-Morozov, Viktor]; Davis, Allen [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=a8ecee8c29c54092b969e9547ea72596-Davis, Allen]; Sasso, Alan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8cb867519abc4dcea88149d12ef3e8e9-Sasso, Alan]; Kapraun, Dustin [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3a53c151b92a472fbfb295ed5df982a7-Kapraun, Du]; Sonja Sax [SSax@ramboll.com]; Ken Mundt [kenneth.mundt@cardno.com]; Miyoung Yoon [Personal Matters / Ex. 6] Kenyon, Elaina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=0395d5b93f214c8ca49066f498f7d5c9-Kenyon, Elaina]
Subject: RE: chloroprene -- in vitro system

Harvey,

First, the blank experiments where Matt measured the system losses in the absence of metabolism show that there is at best minimal non-specific binding, with which the microsomes might compete. When measuring partition coefficients, tissues are homogenized in buffer and it is assumed that the volume of the buffer and the water:air PC can be used to calculate how much of the gas is in the buffer, by mass balance how much is in the tissue. If I assume that the microsome:air PC is the same as liver:air, by the same type of calculation, the extent to which that could impact partitioning from the air to the incubation medium is negligible, since the microsomes are only 0.05% of the medium. To argue with that is to argue with the method by which PCs are estimated.

The data are valid, I am not arguing that. But the data are measurements of gas concentration in the headspace above an incubation mixture, ***not*** direct measurements of the rate of reaction as a function of concentration in the incubation medium. These data are being interpreted, to extract metabolic parameters, using a model that assumes the system is well-mixed and that the metabolic parameters can be estimated based on the amount removed from the headspace, and the water:air partition coefficient; i.e., assuming the same concentration in the entire mixture.

If it were somehow true that the microsomes significantly altered the partitioning, then this computational estimate of concentration in the microsomes where the enzyme is functioning is also wrong, hence the estimated kinetic parameters would be wrong.

I am working under the assumption that Denka paid to collect are also valid data, and are applicable to the in vitro metabolism experiments.

And I don't think we need to make rather stretched hypotheses about what a small amount of microsomes might do to partitioning to explain the discrepancy. But what I do believe that the combined data show is that the computational model being used to interpret the data is incorrect: the data are valid, but there's a problem with the model. In particular, I don't believe you can have the resistance to mass transfer that was measured and complete mixing in a system where metabolism is that high. It only appears to be a discrepancy when you assume the system is well mixed.

I've set up an alternate model where the liquid phase is divided into 10 layers. I just have very preliminary results, need to do some more testing, but I believe it can fit both the Kg data and the metabolic data with a common set of parameters.

On the one hand, you are right that we don't know the exact mixing speed that Matt used, etc. On the other hand, you obtained a Kg which is remarkably, remarkably close to the one I estimated for benzene way back. I don't think that's just a coincidence, but is saying that the resistance to diffusion in water is similar for small VOCs whose molecular weights are not too far apart.

I did suggest trying to fit the Kg to the data, but I didn't think you would get something that much higher than was measured. I don't believe that 0.05% MSP can cause a 20-fold increase in the rate of mass transfer, no way.

When we talked and Jerry showed me the results a few weeks back, I couldn't think of a better explanation. But still, the discrepancy was there… Who is it that said models are most useful when they fail, because that teaches us something? To argue away part of the data just because it makes the model fail is not learning from the failure. I'm sorry it's taken me so long to have this realization about mixing, but it may matter for the interpretation of other data sets where the in vivo metabolism is not rate limiting. And it may matter in estimating the lung parameters, which are key to that metric.

I'm pretty sure that I also suggested that for the data sets which could be fitted with the Kg as measured, you use that Kg, not the one fitted to the liver data. That's not what was done, and I'll want to see if it makes any difference for the lung metabolism simulations.

By the way, in the script I have for the mouse liver metabolism the protein content was set to be 1 mg/ml, but Matt's paper says it was 0.5 mg/ml. So that would impact the IVIVE by a factor of 2, if it wasn't caught/corrected in the latest revision.

It is a shame if no one is collecting such data now, since that would mean using default methods for VOCs where there aren't the data. I thought we still have labs and equipment here where they can be done. Whether it's important enough to do for this particular chemical, or to further our ability to interpret such data for other VOCs is a question. The plots that John Wambaugh shows comparing IVIVE predictions to actual PK data indicate it can be off by an order of magnitude or more. So for chemicals where there are no in vivo data we either build that uncertainty into the risk estimation, or we find a way to reduce it. Improving our ability to interpret in vitro data with a limited set of experiments, even if they can't be done by a contract lab, automated methods, might be worthwhile if it can reduce the prediction error across multiple chemicals.

In the end I believe that if a model is based on assumptions that are invalidated by the data in hand, then the parameters that come from using that model are uncertain, even if the data are valid. In this case the uncertainty may be acceptable, because the in vivo PK is flow limited. But I want to complete a bit more analysis with the multi-layer model before convincing myself of that.

-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Wednesday, June 05, 2019 6:34 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen Personal Matters / Ex. 6 Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon Personal Matters / Ex. 6 ; Kenyon, Elaina <Kenyon.Elaina@epa.gov>
Subject: RE: chloroprene -- in vitro system

Hi Paul
, to investigate your hypothesis regarding diffusion limitation in the liquid phase. However, even though the experiment you describe may sound simple, performing it correctly would be just as difficult as the original studies conducted by Matt Himmelstein. Unfortunately, there is no longer anyone conducting these kinds of studies. Both John Wambaugh at NCCT and I have tried to identify laboratories with experience in conducting in vitro metabolism studies with volatiles, but we have both been unsuccessful. That is why Denka had to use an environmental contract laboratory to conduct the Kg study.

I have discussed this question with Miyoung Yoon, who is now at FDA, and it was she who suggested that the presence of microsomes in Matt's studies would have greatly increased the availability of chloroprene for metabolism by competing with other sources of non-specific binding. She is the most experienced researcher in the area of *in vitro* metabolism that I know of. I'm afraid this difference in opinion will need to go unresolved, however, not only because the necessary studies are impractical but also because the relevance of any new results to Matt's published studies would be highly uncertain. The difficulty is that Kg is just an empirical parameter that represents the rate of mass transfer under specific experimental conditions. Most importantly, as mixing is increased, the transition from simple diffusion to laminar convection and then to turbulent convection impacts the rate of mass transfer in a nonlinear manner, so extrapolation from one experiment to another would be extremely difficult.

I have also discussed this question with Mel Andersen, and he believes that the published *in vitro* data are completely reliable. He agrees with the approach you suggested for estimating Kg from the male mouse liver metabolism data by fixing Km at the value of 1 uM supported by the literature on cyp2E1 substrates. Re-estimating the metabolism parameters with the estimated Kg results in a 25% decrease in risk compared to using the published values. Values of Kg lower than the value estimated from the metabolism data would reduce risk estimates even further. I just don't see the benefit of performing any additional studies.

With kind regards
Harvey Clewell
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From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Wednesday, June 5, 2019 9:57 AM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen <Personal Matters / Ex. 6>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon <Personal Matters / Ex. 6>; Kenyon, Elaina <Kenyon.Elaina@epa.gov>
Subject: RE: chloroprene -- in vitro system

I've changed the subject to better reflect the topic.

This morning I realized there's actually a fairly simple experiment that could determine if my hypothesis on diffusion limitation in the liquid phase is correct: run incubations with ½ (or less) of the total incubation mixture, concentrations of microsomes, etc, otherwise the same. (We'd want to have parallel experiments, same lab, same microsomes, etc., with full volume.)

If the system is well mixed, as the current model suggests, then the rate at which chloroprene is removed from the headspace (mass/time) would be reduced by ½, since there's ½ the microsomes doing the work. You'd have to incorporate the change in head-space volume in the calculation (either way).

Alternately, if there is diffusion limitation, with the microsomes near the surface doing the bulk of the metabolism, then the rate of chloroprene removal would not be reduced by ½. A higher fraction of the microsomes would be near the air:liquid interface, so the rate of removal per mg microsome in the system would be higher.

Since the concentration in the incubation solution is effectively calculated by mass balance, this would also lead to an increase in the estimated concentration associated with a given rate of metabolism, I'm pretty sure. The result would then also be an increase in the apparent Km.

Doing these experiments would then evaluate the extent to which mass transport in the liquid phase is limiting in this system, using live/active microsomes, doesn't require any more elaborate analytic methods than already employed.

-Paul

From: Schlosser, Paul
Sent: Tuesday, June 04, 2019 5:14 PM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen <Personal Matters / Ex. 6>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John

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Personal Matters / Ex. 6 >
Subject: RE: chloroprene -- Bayesian analysis

Harvey, all,

In the Kg experiments, if the sampling of the liquid phase is well into the liquid, away from the air:liquid interface, but mixing is sufficient to keep microsomes evenly distributed, then it's possible for the CP concentration near the surface to be higher, less limited by mass transfer resistance, than in the middle or bottom. If the microsomes near the surface are responsible for the majority of the metabolism, then that could explain the discrepancy between the Kg data/model and the metabolic data.

But that would also mean that the activity of those microsomes was higher than currently estimated… if only 10% of the microsomes (those near the surface) are responsible for the metabolism, the actual Vmax would be 10x higher per mg microsomes, for example. Since the in vivo PK are flow limited, the fits to those data would be the same, if Vmax (in the liver) is actually 10x higher, those in vivo data don't invalidate this hypothesis.

The incubation results would still be linear with microsome content by this explanation, presuming they are well mixed. Using ½ the total microsomes would put that much less near the surface, resulting in a proportional decrease in removal from the gas phase. It's saying that under conditions of high metabolic activity, the assumption of a well-mixed incubation volume is not valid. I think that's more likely than a small fraction of microsomes significantly affecting transport through the entire volume.

Good evening, until tomorrow!

-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Tuesday, June 04, 2019 2:12 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen Personal Matters / Ex. 6 Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon
Personal Matters / Ex. 6 >
Subject: RE: chloroprene -- Bayesian analysis

Hi Paul

I do not agree that the apparent discrepancy between the Kg experiments and the metabolism experiments leads to parameter uncertainty. To break the collinearity between Km and Kg, we have followed your suggestion of fixing Km at a value based on the literature for cyp2e1 substrates (1 uM), and have re-estimated Vmax and Kg in the male mouse liver, which shows the highest rates of metabolism. The resulting value of Kg represents the maximum limitation on transport in the in vitro studies that is consistent with the data. It does not demonstrate, however, that there was any significant transport limitation in those studies.

Personally, I have complete confidence in the metabolism data collected by Matt Himmelstein and in the approach he used for its analysis. I see no evidence to support an assumption of transport limitation in his studies. The constants derived in the published analysis are consistent with a large body of work on *in vivo* estimation of kinetic constants for clearance of well-metabolized vapors (i.e., with relatively low Km values). Moreover, assuming that there was a significant limitation on transport in these studies results in Km values that are implausibly low, which in turn results in lower risk estimate compared to use of the published values.

The discussion below of the role of plasma proteins on metabolism is from James Gillette's 1973 paper in the Annals of the NY Academy of Science. He suggested that binding proteins in the plasma can accelerate metabolism by acting as carriers of a drug to the vicinity of the hepatocytes. I believe that microsomal proteins can play a similar role in the *in vitro* studies: in the presence of mixing, non-specific binding of a lipophilic compound to proteins can serve to overcome the transport limitation associated with penetrating the aqueous media. In other words, since chloroprene is lipophilic, diffusion through the aqueous media in the *in vitro* assay would normally be rate-limiting, but if the media is well-mixed and contains microsomal proteins, then non-specific binding of chloroprene to the microsomes could greatly enhance its availability for metabolism.

Unfortunately, investigating this effect in the *in vitro* system would not be at all straightforward, because the microsomal proteins serve both as the site of metabolism and as a source of non-specific binding that competes with the surface of the vial. In fact, I do not believe it is possible to experimentally determine a Kg that would be appropriate in any microsomal metabolism study, because of the dual role that the microsomes play (metabolism and non-specific binding). Denaturing the microsomal proteins in order to eliminate metabolism would also alter their tertiary binding structure.

it is impossible to predict when the dissociation of the drug-protein complex is rate limiting from the association constants alone. It seems probable, however, that the rate of dissociation of the drug-protein complex seldom becomes rate limiting in the metabolism of drugs.

It seems more probable that diffusion of the free drug from the plasma into hepatocytes may be the rate-limiting step in the metabolism of drugs by highly active enzymes in the liver. When this occurs, the bound form of the drug in plasma remains in equilibrium with its free form as the concentration of unbound drug in plasma declines during the passage of the blood through the hepatic sinusoids. However, it is also possible that drug-binding proteins in the cytoplasm of hepatocytes hasten metabolism by maintaining the concentration gradient across the membranes of the hepatocytes and by increasing the amount of drug available to the enzymes located toward the central parts of the cells. Indeed, Y and Z proteins [18–21] are thought to act as carriers of sulfobromophthalein and other anions. But, whether they actually act as carriers or as tissue stores of the anions depends on a number of factors that include the association constants and the concentrations of the proteins as well as on the maximum rate and the K_m values of the drug-metabolizing enzymes and the relative diffusivities of the unbound drug and drug-protein complexes. Because of the complexities of these interrelationships, it is frequently difficult to determine when binding sites in hepatocytes serve as transport mechanisms or as storage mechanisms.

To determine whether plasma proteins act as transport carriers, it is necessary to calculate the apparent clearance on the basis of the concentration of unbound drug in plasma and to compare these apparent clearance values (rate of elimination/concentration of unbound drug) with hepatic blood flow rate. If the apparent clearance exceeds the hepatic blood flow when the drug is known to be eliminated solely by the liver, it may be concluded that the plasma proteins or other components in blood act as transport carriers. When it is not known whether a drug is eliminated solely by the liver, it would be useful to determine whether the clearance value exceeds the cardiac output, because the true clearance can never exceed the cardiac output.

With kind regards

Harvey Clewell
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From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Friday, May 31, 2019 11:41 AM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry

<rgentry@ramboll.com>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>
Subject: RE: chloroprene -- Bayesian analysis

Harvey, all,
(Adding Dustin Kapraun: I'll catch you up later.)

Food for thought for June 12:

Regarding the in vitro analyses, the apparent discrepancy between the "Kg" experiments and the metabolic experiments leads to parameter uncertainty. Kg and the Km values can't be estimated independently from only the metabolic experiments, as you state in the manuscript. But to fully estimate the impact of that uncertainty in subsequent risk estimation, one could potentially use the results of a Bayesian analysis, not just the mean values, but the parameter distributions.

So the first estimation of Kgi and P (from data without microsomes) resulted in a joint distribution of these parameters. Some of those data are consistent with a higher P and Kgi than the mean values – the upper data in Figure B-1. Likewise your review of the literature on Km values effectively provides an informed prior on that parameter. Instead of fixing one or the other of these, formal Bayesian analysis could use those as priors when analyzing the data with active microsomes. I wonder if there are values of Kgi and P consistent with the upper end of the Kg-data (ie, within the uncertainty given those data) that are also consistent with the metabolic data?

I presume the Kg experiments, like the metabolic experiments, involved repeated measures, which needs to be properly accounted for in setting up the likelihood calculation in order to estimate the true uncertainty, full possible range of parameters. The number of independent experiments in Figure B-1 is a lot less than the number of data points, yes? (Since there are clusters of pink points at each time point, it's more than the number of colors used.) The estimated parameter uncertainty estimate would be too low if all the data are treated as independent. Likewise for the metabolic experiments.

If it wasn't done this way (accounting for repeated measures in the likelihood; formal Bayesian sequential parameter estimation), how hard would it be? To unpack this fully, and consider options, we may need to have original data by experimental unit (incubation vial), if it's not already set up that way.

-Paul

From: Schlosser, Paul
Sent: Thursday, May 30, 2019 8:06 AM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: chloroprene

Harvey, all,
(Copying EPA folk)

In the manuscript, you suggest that binding to the microsomal protein, which wasn't present in the Kg-measurement experiments, could have altered the partitioning between air and liquid phases, thereby resulting in the changed mass transfer. If true, this could be explained in the model by changing the water:air partition coefficient. Maybe you can test the hypothesis that way ahead of our meeting, so we know if it's a valid explanation or not. The microsomal concentration was 0.5 mg/ml, ~0.05%, so I don't know that it could alter partitioning too much, but it would be good to know ahead of the 12th if changing the PC alone to any extent could explain the apparent discrepancy.

-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Tuesday, May 14, 2019 4:40 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>
Subject: chloroprene

Hi Paul

Here is the revised manuscript on the chloroprene PBPK model, plus all of the supplemental materials that can be sent via email. The R model and two additional supplemental files (the IISRP report on the in vivo study and the Teklab report on the Kg study) will be transmitted separately, but I don't think you will really need to look at them at this point.

I'm going to be in Netherlands next week for Alina Efremenko's PhD ceremony, so it would be great if we could get together sometime this week to talk about the new analyses documented in the paper. Would that be possible? Jerry and I are free pretty much any time.

With kind regards
Harvey Clewell
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Message

From: Schlosser, Paul [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=121CF759D94E4F08AFDE0CEB646E711B-SCHLOSSER, PAUL]
Sent: 10/29/2018 12:34:36 PM
To: HIMMELSTEIN, MATTHEW W [Matthew.W.Himmelstein@dupont.com]
CC: Davis, Allen [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=a8ecee8c29c54092b969e9547ea72596-Davis, Allen]; Sasso, Alan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8cb867519abc4dcea88149d12ef3e8e9-Sasso, Alan]; Vandenberg, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=dcae2b98a04540fb8d099f9d4dead690-Vandenberg, John]; Thayer, Kris [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3ce4ae3f107749c6815f243260df98c3-Thayer, Kri]; Bahadori, Tina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7da7967dcafb4c5bbc39c666fee31ec3-Bahadori, Tina]; Harvey Clewell [HClewell@ramboll.com]; Jerry Campbell [JCampbell@ramboll.com]; Robinan Gentry [rgentry@ramboll.com]; cvanlandingham@ramboll.com [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=usereda39e51]; Sonja Sax [SSax@ramboll.com]
Subject: RE: Chloroprene In Vitro model

Matt,

Personal Matters / Ex. 6 I heard from Harvey that they are working on a contract to measure the gas-liquid mass transfer coefficient, but it would still be helpful to have the lab report from the initial in vitro studies, as we can also check the data against those, and the headspace sample volumes.

Thanks,
-Paul

From: HIMMELSTEIN, MATTHEW W [mailto:Matthew.W.Himmelstein@dupont.com]
Sent: Thursday, October 04, 2018 6:45 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Harvey Clewell <HClewell@ramboll.com>
Cc: Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Jerry Campbell <JCampbell@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; cvanlandingham@ramboll.com; Sonja Sax <SSax@ramboll.com>
Subject: RE: Chloroprene In Vitro model

Sorry folks,

Personal Matters / Ex. 6

Matt

Matthew Himmelstein
DuPont Haskell Global Centers
Phone 302 451 4537

From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Wednesday, October 03, 2018 2:12 PM
To: Harvey Clewell <HClewell@ramboll.com>

Cc: Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Jerry Campbell <JCampbell@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; cvanlandingham@ramboll.com; Sonja Sax <SSax@ramboll.com>; HIMMELSTEIN, MATTHEW W <Matthew.W.Himmelstein@dupont.com>
Subject: [EXTERNAL] RE: Chloroprene In Vitro model

Harvey,

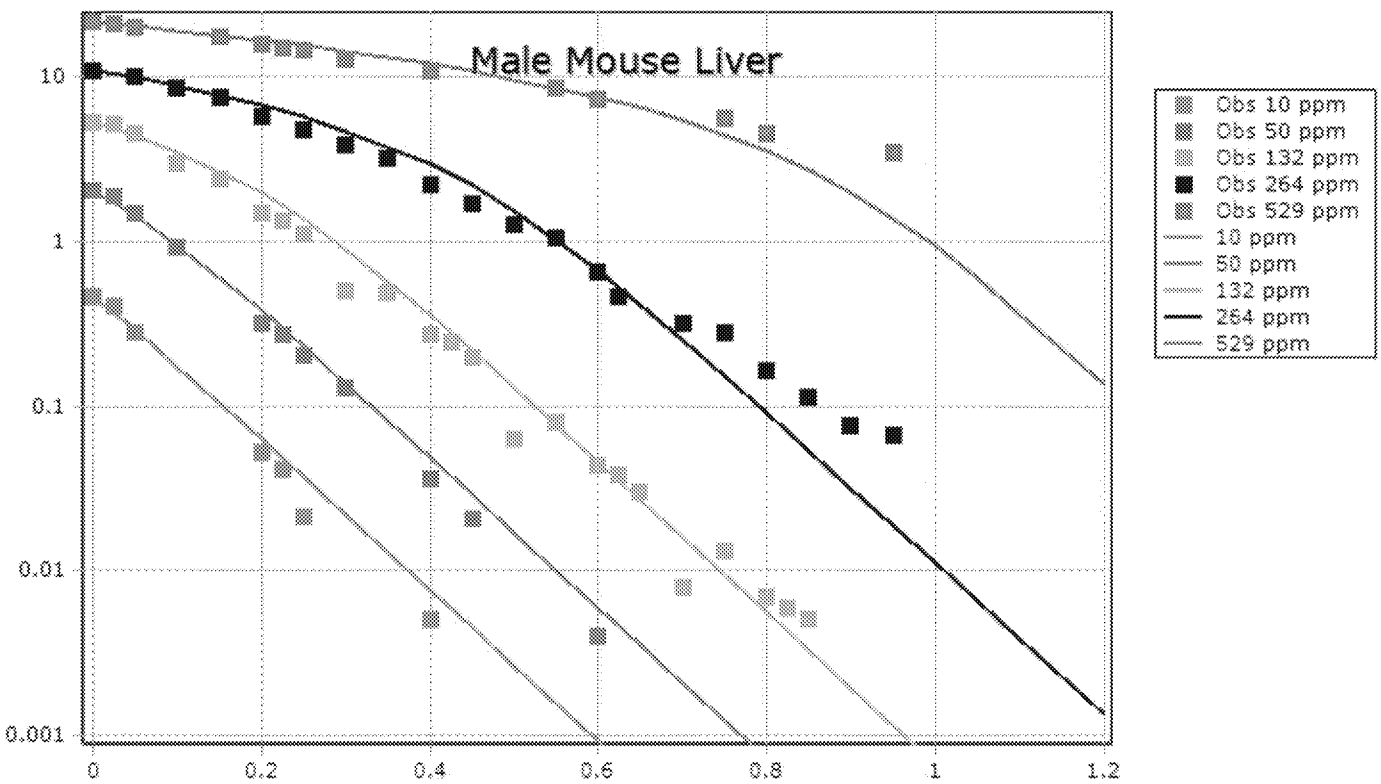
It struck me as unlikely that the Km values would differ by a factor of 400-500 between liver and lung, though some difference could occur due to a different ratio of enzymes in each tissue.

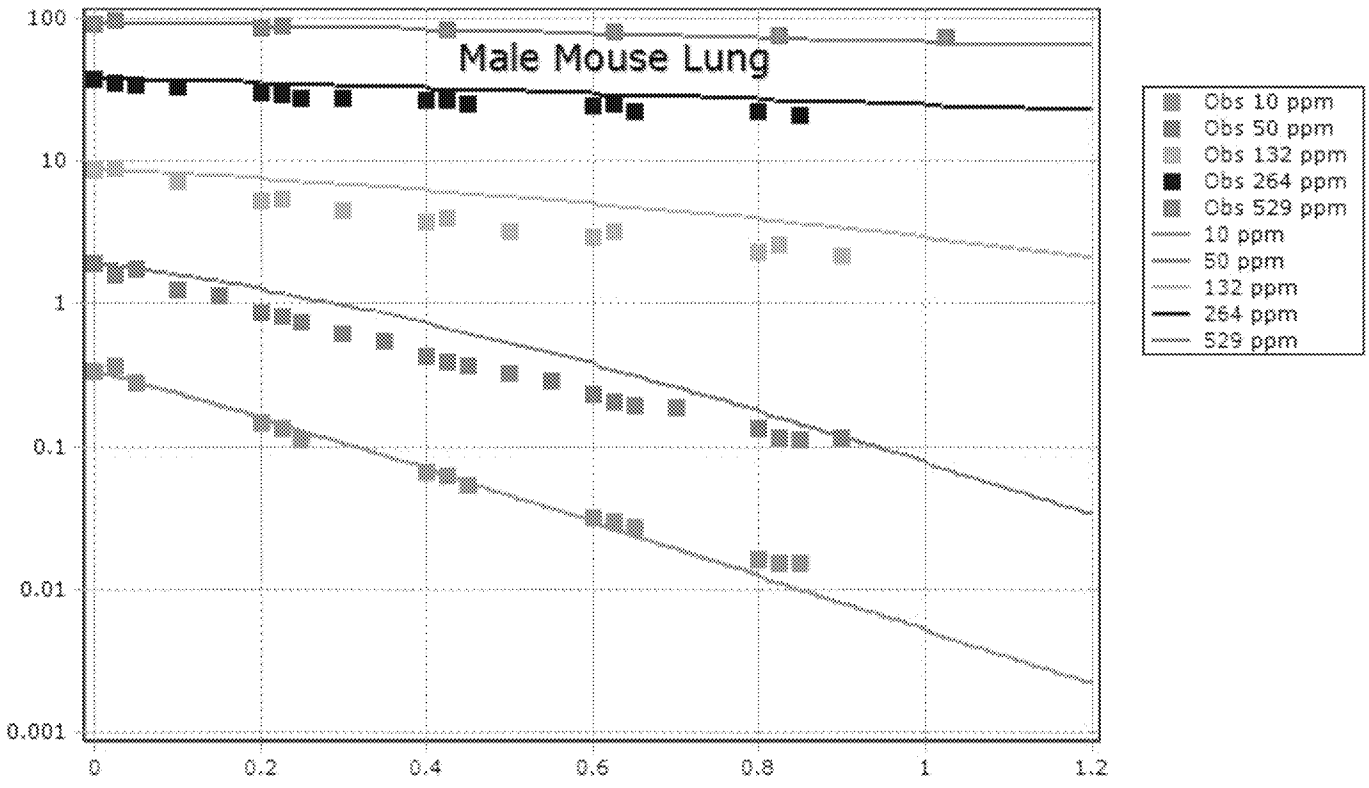
Looking at the male mouse data, one interpretation is that the Km for the liver is somewhere in between 1.36 uM (Yuching's value) and 0.0028, depending on KG. This includes the values of Km obtained from fitting the male mouse lung data, with this air-liquid term in place. Below is rough, eyeball fitting, but with a value of KG = 0.2 and common value of Km = 0.7 (and assuming that VINJ = 0.004 for the male mouse lung experiments, same as liver, but that's not too big of a factor on this scale). I expect that with formal optimization the fits could be improved.

On the one hand, the results so far do suggest that the uncertainty here doesn't have a large impact on the in vivo predictions. But this also indicates that the data available aren't sufficient to say that the Km is really different between the tissues evaluated, and a way to possibly reduce the uncertainty among all the parameters is to re-fit the KG, tissue-specific Vmax values, and Km simultaneously to liver, lung, and kidney data; i.e., with a fixed Km.

__However__, I don't suggest going anywhere with that until we get the report from Matt, to resolve the question of the sample volume for the male lung and liver. I recall that it was set at ~ 4 uL in the code in the report Matt sent from the 2nd set of studies (Yuching's code), for simulations of the older data, and while it doesn't show much on the log scale, it makes a difference on linear scale, will impact the optimization I believe.

-Paul





From: Harvey Clewell [mailto:HClewell@ramboll.com]
Sent: Wednesday, October 03, 2018 11:41 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Jerry Campbell <JCampbell@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; cvanlandingham@ramboll.com; Sonja Sax <SSax@ramboll.com>; HIMMELSTEIN, MATTHEW W <Matthew.W.Himmelstein@dupont.com>
Subject: RE: Chloroprene In Vitro model

Sorry Paul, I forgot to mention that these comparisons were run with updated in vitro to in vivo scaling parameters recommended by Miyoung Yoon based on her review of information on protein content that's available now as compared to when Yang et al. (2012) was written. I mentioned that she was doing this when I gave the presentation at EPA and she's now completed it. So you can't compare the results I sent you with the results using the original Yang et al. (2012) scaling. If you'd like we can run the analysis again with the old scaling parameters.

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From: Schlosser, Paul <Schlosser.Paul@epa.gov>
Sent: Wednesday, October 3, 2018 11:31 AM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Jerry Campbell <jcampbell@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; Cynthia Van Landingham <cvanlandingham@ramboll.com>; Sonja Sax <ssax@ramboll.com>; HIMMELSTEIN, MATTHEW W

<Matthew.W.Himmelstein@dupont.com>
Subject: RE: Chloroprene In Vitro model

Harvey,

Something is odd with the results…

First, small thing, the lowest exposure should be 12.8 ppm, not 12.3.

But more significantly, when I run Female_mouse_dose_metric_2.R and _3.R, I get the following:

_2.R

	ppm	AMP	AMPLU	AMPK
1	12.3	1.487844	0.7019859	0.003269034
2	32.0	3.910366	1.1611204	0.008441814
3	80.0	9.739528	1.5521656	0.020442855

_3.R

	ppm	AMP	AMPLU	AMPK
1	12.3	1.473707	0.9477216	0.001063307
2	32.0	3.888278	1.5712141	0.001577733
3	80.0	9.582979	2.1074212	0.001958602

These match almost exactly to the spreadsheet values you sent in August, but differ quite a bit from the ones sent yesterday, "Yang et al. 2012 Table 3 Estimated Metabaolic Point Est. Used" for the female mouse.

Then, running the corresponding male mouse scripts:

_2.R – these numbers are a bit off of what was in the August 3rd spreadsheet. For example, that lists AMPK(80 ppm) = 0.558

	ppm	AMP	AMPLU	AMPK
1	12.3	1.198161	3.797067	0.3334743
2	32.0	3.448833	6.485935	0.4666568
3	80.0	9.298214	8.621115	0.5440947

_3.R – these match the Aug 3 spreadsheet pretty well, just some off by +/- 0.001.

	ppm	AMP	AMPLU	AMPK
1	12.3	1.101225	5.001708	0.3441541
2	32.0	3.286599	8.565978	0.4547611
3	80.0	9.093323	11.280996	0.5152051

But again, quite different from results sent yesterday (left-most set).

It still seems crazy that the total metabolism in the male lung is greater than in the whole liver! But that aside, the baseline tables sent yesterday seem to be off.

-Paul

From: Harvey Clewell [mailto:HClewell@ramboll.com]
Sent: Tuesday, October 02, 2018 5:27 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Jerry Campbell <JCampbell@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; cvanlandingham@ramboll.com; Sonja

Sax <SSax@ramboll.com>; HIMMELSTEIN, MATTHEW W <Matthew.W.Himmelstein@dupont.com>
Subject: RE: Chloroprene In Vitro model

HI Paul

We did check the lung and kidney too. Here's a spreadsheet with all the calculations. The dose metric comparisons I sent you were with all the tissue metabolism parameters re-estimated using the kg estimated from the male mouse liver data. I imagine the effect of kg will be less in the rat than the mouse, but we can check that.

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From: Schlosser, Paul <Schlosser.Paul@epa.gov>
Sent: Tuesday, October 2, 2018 4:06 PM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Jerry Campbell <jcampbell@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; Cynthia Van Landingham <cvanlandingham@ramboll.com>; Sonja Sax <ssax@ramboll.com>; HIMMELSTEIN, MATTHEW W <Matthew.W.Himmelstein@dupont.com>
Subject: RE: Chloroprene In Vitro model

Harvey,

I had thought about doing something like this but was concerned about the identifiability. The Km that you get is *so* low that I suspect the real kg must be higher. When I tested kg = 10x the value from the benzene studies, the Km (eyeball fitting) came out to only ½ the original value, so it's probably really the ratio of the two that we can identify.

That being said, I think what you have here is probably a lower bound on the Km, so if the final model predictions aren't really affected, then the results of this approach can be a bounding sensitivity analysis.

But I think the next question is what happens when this value of kg (and Km) is applied to the mouse lung metabolism data? (And what about the rat?) Perhaps those rates are low enough that it doesn't matter, but that also needs to be determined. Since lung metabolism isn't limited by blood flow, I'd expect a change in the metabolic parameters to have more of an effect on that estimate.

They do still run in vitro metabolism studies in NHEERL, but that's a different lab/center, they have their research plan and budget to consider, etc.

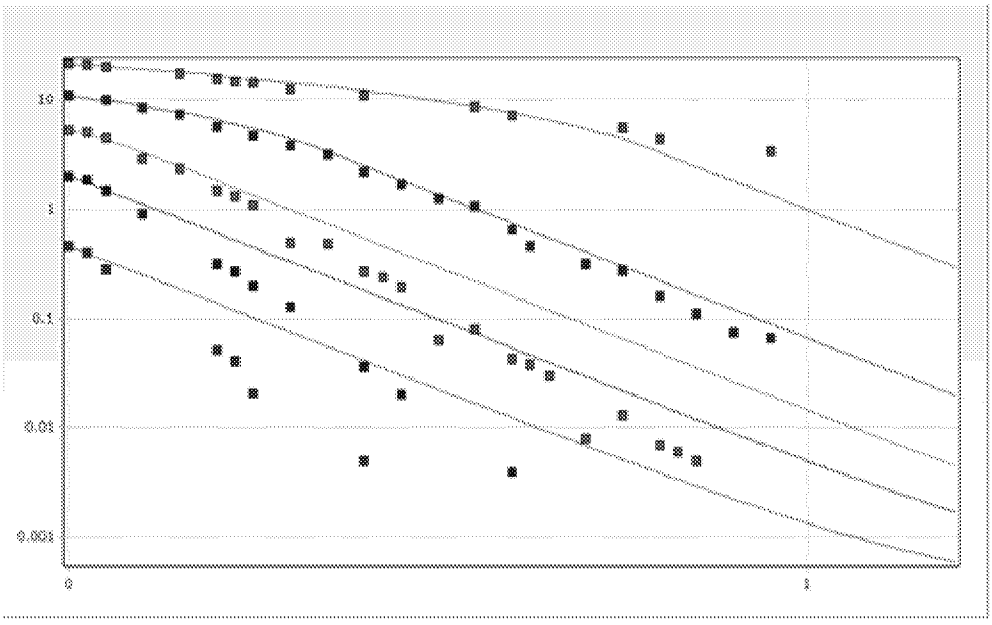
-Paul

From: Harvey Clewell [mailto:HClewell@ramboll.com]
Sent: Tuesday, October 02, 2018 3:27 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Jerry Campbell <JCampbell@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; cvanlandingham@ramboll.com; Sonja Sax <SSax@ramboll.com>; HIMMELSTEIN, MATTHEW W <Matthew.W.Himmelstein@dupont.com>
Subject: RE: Chloroprene In Vitro model

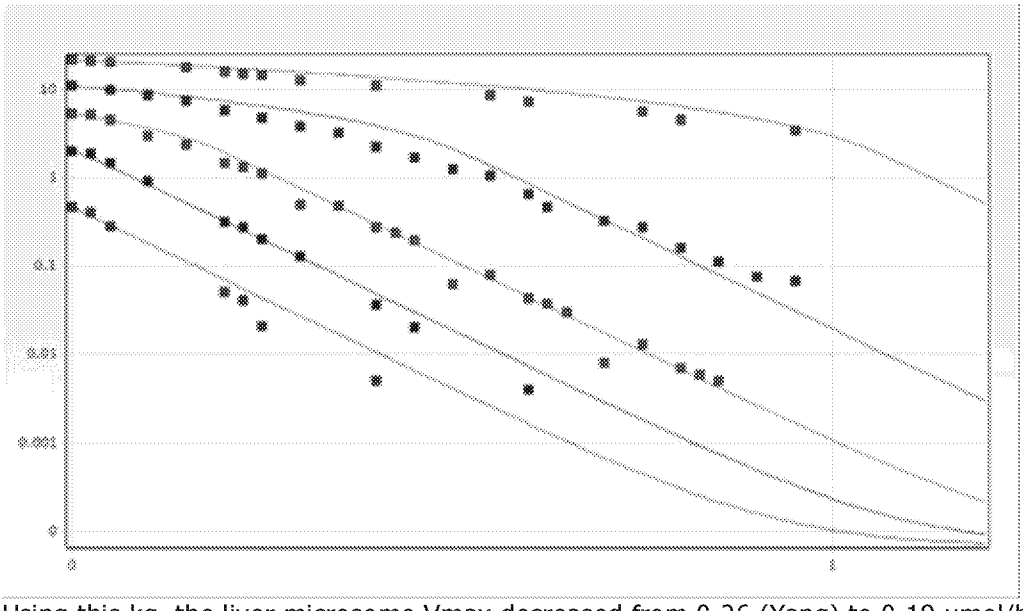
Hi Paul

We've performed an analysis to estimate the potential impact of the use of an equilibrium assumption in the estimation of the in vitro metabolism parameters. Based on this analysis I don't believe it is necessary to conduct new studies to determine a kg for chloroprene. The male mouse liver data are adequate to inform the value of this parameter. Moreover, I'm concerned that such a study would be difficult to perform since the research laboratories that used to conduct such studies with volatiles (WPAFB, CIIT, CSU, Hamner) are no longer available. Does NHEERL still have the capability to perform such a study?

We began, as you did, with the male mouse liver, since that's the strongest data (highest metabolism rates). Scaling the kg from your benzene study (0.434 mL/min= 0.026 L/h) by the ratio of the surface areas in the 4 mL vials you used vs. the 10 mL vials Matt used results in a kg = 0.0636 L/hr and kl = kg/P(liquid/air), which is too low to fit the rate of metabolism at low concentrations.



Next, we used the Nelder-Mead optimization to jointly estimate Vmax, Km, and kg with kl set equal to kg/PC. The resulting estimate of kg was 0.100 L/hr, about 50% higher than the value estimated by scaling your benzene data.



Using this kg, the liver microsome Vmax decreased from 0.26 (Yang) to 0.19 umol/hr/mg protein and the Km decreased from 1.36 to 0.0028 uM. This is again consistent with what you found.

In order to evaluate the potential effect of kg on model predictions we used the kg estimated from the male mouse liver data to re-estimate the metabolism parameters for the liver, lung and kidney in the female mouse. The resulting dose metrics for the female mouse lung are not affected by the use of kg.

Yang et al. 2012 Table 3 Estimated Metabaolic Point Est. Used

Female Mouse Initial Parms			
PPM	AMP	AMPLU	AMPK
12.3	0.60	0.42	0.0015
32	1.60	0.66	0.0022
80	4.04	0.86	0.0027

Benzene KG Adjusted for Vial Surface Area

Female Mouse Initial Parms			
PPM	AMP	AMPLU	AMPK
12.3	0.61	0.42	0.0016
32	1.61	0.67	0.0017
80	4.08	0.86	0.0019

KG Optimized to Male Mouse Liver In Vitro

Female Mouse Initial Parms			
PPM	AMP	AMPLU	AMPK
12.3	0.60	0.41	0.0016
32	1.60	0.66	0.0018
80	4.07	0.86	0.0019

In retrospect, it makes sense that kg doesn't affect the model predictions since the only tissue where metabolism is sufficiently rapid to make kg rate-limiting is the liver, but at the bioassay concentrations liver metabolism is blood-flow limited so decreasing km does not increase the rate of liver metabolism.

Harvey Clewell

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From: Schlosser, Paul <Schlosser.Paul@epa.gov>
Sent: Wednesday, September 19, 2018 2:14 PM
To: HIMMELSTEIN, MATTHEW W <Matthew.W.Himmelstein@dupont.com>; Jerry Campbell <jcampbell@ramboll.com>
Cc: Harvey Clewell <HClewell@ramboll.com>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>
Subject: RE: Chloroprene In Vitro model

I'm sending again, including John Vandenberg, Kris Thayer, and Tina Bahadori.

-Paul

From: Schlosser, Paul
Sent: Wednesday, September 19, 2018 1:39 PM
To: 'HIMMELSTEIN, MATTHEW W' <Matthew.W.Himmelstein@dupont.com>; 'Jerry Campbell' <JCampbell@ramboll.com>
Cc: 'Harvey Clewell' <HClewell@ramboll.com>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: Chloroprene In Vitro model

Matt, all,

I'm following up to see how things stand regarding the search for additional data. In a separate note Harvey said there should be a report (IISRP?) for the earlier in vitro studies, which it could help to have. Please send any that you have.

As it stands, we have mostly halted our QA review, as it strongly hinges on the equilibration assumption in the in vitro modeling. The code for the in vitro and in vivo models has checked out, issues resolved, and I think all other parameter discrepancies have been resolved – a few changes but none that should make a really large difference.

I realize it might take some time for files to be retrieved from archives and reviewed, but it's now been a couple of weeks since I provided the written details on what we are seeking. Can you tell us where things stand on your end?

The simulations I've run/provided show that the fits to the low concentration in vitro data depend significantly on the assumption that gas-liquid equilibration is not rate limiting, and the data are consistent with the possibility that it is a factor, requiring a fairly large revision in the estimated Km value(s). As is, my conclusion is that there is uncertainty due to the lack of data on the mass transfer rate, and there isn't an easy way that I can think of (or that we are likely to undertake ourselves) for estimating or bounding that uncertainty. The model results are too uncertain to use, given the data and assumptions.

If data are obtained (from archives or newly developed) that show that mass transfer is a factor, it will then be up to Denka/Ramboll to revise the in vitro parameter estimation accordingly, and propagate that into the in vivo model, before we would continue our QA.

As indicated in previous emails, our QA will also involve comparing model predictions to the nose-only in vivo PK data from 2004: the model should be able to fit with parameters adjusted in a way consistent with the hypothesis that there may be an effect of the exposure system on respiration, but this would not be exposure-concentration-dependent. That will require creating model scripts to run these simulations and compare model outputs to the data. While we are prepared to do that work as part of our QA, provided that the mass transfer data become available, we are not planning to begin that work until those data are available and any necessary revisions of the in vitro modeling have been completed. Alternately, Ramboll colleagues could create the scripts in the meantime, which would speed up the QA.

Sincerely,
-Paul

~~~~~~~~~~~~~~~~~~~~~~~~
Paul M. Schlosser
NCEA, U.S. EPA
M.D. B243-01
RTP, NC 27711
T: 919-541-4130
F: 919-685-3330
E: schlosser.paul@epa.gov
~~~~~~~~~~~~~~~~~~~~~~~~

From: Schlosser, Paul
Sent: Wednesday, September 05, 2018 12:06 PM
To: 'HIMMELSTEIN, MATTHEW W' <Matthew.W.Himmelstein@dupont.com>; Jerry Campbell <JCampbell@ramboll.com>
Cc: Harvey Clewell <HClewell@ramboll.com>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: Chloroprene In Vitro model

Matt,

Sorry. I was also wondering at the volume being 1.6 mL bigger than advertised, it seemed like a large discrepancy.

A memo is attached, but here is what I've gotten from looking at the code in the appendix of the report you sent:

- Data to indicate that mass transfer resistance is not significant are still lacking.

- The sample volume (VINJ) for all the CP ***oxidation*** experiments in the 2004 paper should be ~ 400 uL, including male mouse and rat liver and lung data. But the code in the report uses 385.8 uL for male data and exactly 200 uL for male data. Is the higher accuracy for the rodent male and human data supported by some measurements?

- Assuming a similar accuracy, the vial volume (VVIAL) for all experiments described in the 2004 paper should be 0.0120 L. This value should be used for male mouse and rat liver and lung data. (We'll use 0.0116 L for the female mouse and rat data and the kidney data.)

Thanks,
-Paul

Message

From: Harvey Clewell [HClewell@ramboll.com]
Sent: 10/26/2018 4:54:56 PM
To: Schlosser, Paul [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=121cf759d94e4f08afde0ceb646e711b-Schlosser, Paul]
Subject: RE: chloroprene

I have to admit I miss working in a lab.

Harvey Clewell
Principal Consultant

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From: Schlosser, Paul <Schlosser.Paul@epa.gov>
Sent: Friday, October 26, 2018 12:49 PM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <jcampbell@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; Sonja Sax <ssax@ramboll.com>; Davis, Allen <Davis.Allen@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>
Subject: RE: chloroprene

No, I have not. **Personal Matters / Ex. 6**

Personal Matters / Ex. 6 you could have just walked down the hall, eh? ☺

-Paul

From: Harvey Clewell [mailto:HClewell@ramboll.com]
Sent: Friday, October 26, 2018 11:36 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; Sonja Sax <SSax@ramboll.com>
Subject: chloroprene

Hi Paul

Have you heard back from Matt yet? We've been looking onto the possibility of having a study done to determine the kg for chloroprene in Matt's in vitro assays and we've found a potential contract lab. When we get a study protocol from the laboratory, I'll to send it to you for your review.

Harvey Clewell

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hclewell@ramboll.com

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Message

From: Schlosser, Paul [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=121CF759D94E4F08AFDE0CEB646E711B-SCHLOSSER, PAUL]
Sent: 10/4/2018 11:58:48 PM
To: HIMMELSTEIN, MATTHEW W [Matthew.W.Himmelstein@dupont.com]; Harvey Clewell [HClewell@ramboll.com]
CC: Davis, Allen [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=a8ecee8c29c54092b969e9547ea72596-Davis, Allen]; Sasso, Alan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8cb867519abc4dcea88149d12ef3e8e9-Sasso, Alan]; Vandenberg, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=dcae2b98a04540fb8d099f9d4dead690-Vandenberg, John]; Thayer, Kris [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3ce4ae3f107749c6815f243260df98c3-Thayer, Kri]; Bahadori, Tina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7da7967dcafb4c5bbc39c666fee31ec3-Bahadori, Tina]; Jerry Campbell [JCampbell@ramboll.com]; Robinan Gentry [rgentry@ramboll.com]; cvanlandingham@ramboll.com [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=usereda39e51]; Sonja Sax [SSax@ramboll.com]
Subject: RE: Chloroprene In Vitro model

Thanks for letting us know, Matt. That's an important job too. I think we have a plan and can be prepared for when you can get back to us.

-Paul

From: HIMMELSTEIN, MATTHEW W [mailto:Matthew.W.Himmelstein@dupont.com]
Sent: Thursday, October 04, 2018 6:45 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Harvey Clewell <HClewell@ramboll.com>
Cc: Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Jerry Campbell <JCampbell@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; cvanlandingham@ramboll.com; Sonja Sax <SSax@ramboll.com>
Subject: RE: Chloroprene In Vitro model

Sorry folks,

I a
w

Personal Matters / Ex. 6

Matt

Matthew Himmelstein
DuPont Haskell Global Centers
Phone 302 451 4537

From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Wednesday, October 03, 2018 2:12 PM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Jerry Campbell <JCampbell@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; cvanlandingham@ramboll.com; Sonja

Sax <SSax@ramboll.com>; HIMMELSTEIN, MATTHEW W <Matthew.W.Himmelstein@dupont.com>
Subject: [EXTERNAL] RE: Chloroprene In Vitro model

Harvey,

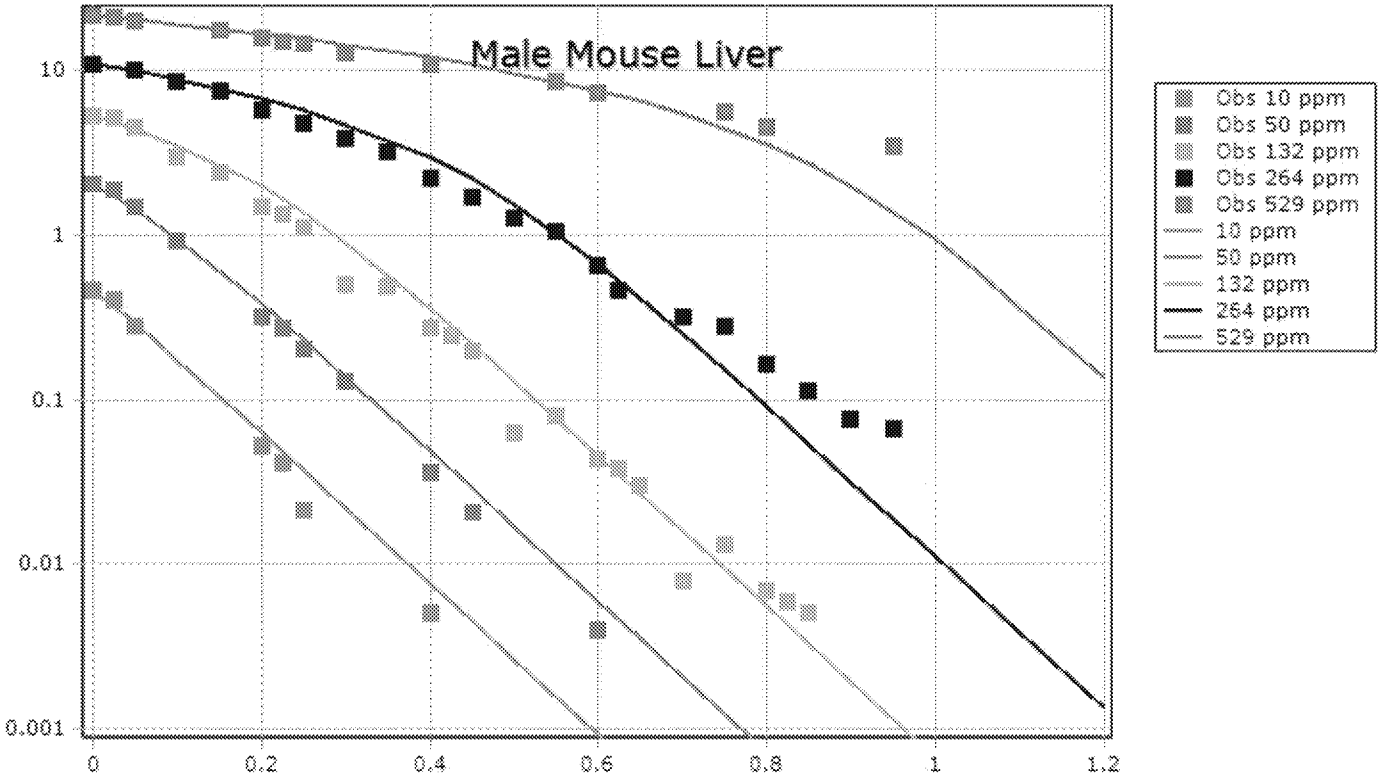
It struck me as unlikely that the Km values would differ by a factor of 400-500 between liver and lung, though some difference could occur due to a different ratio of enzymes in each tissue.

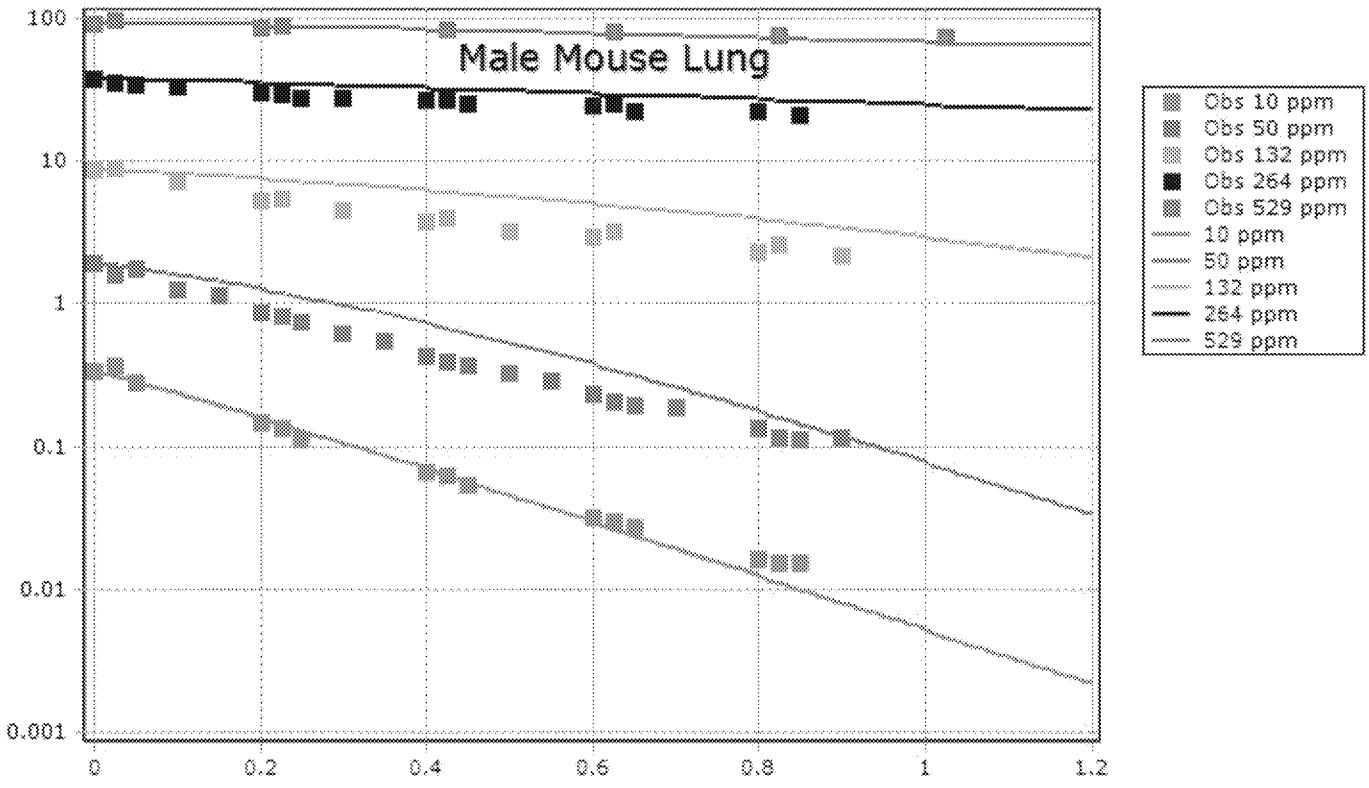
Looking at the male mouse data, one interpretation is that the Km for the liver is somewhere in between 1.36 uM (Yuching's value) and 0.0028, depending on KG. This includes the values of Km obtained from fitting the male mouse lung data, with this air-liquid term in place. Below is rough, eyeball fitting, but with a value of KG = 0.2 and common value of Km = 0.7 (and assuming that VINJ = 0.004 for the male mouse lung experiments, same as liver, but that's not too big of a factor on this scale). I expect that with formal optimization the fits could be improved.

On the one hand, the results so far do suggest that the uncertainty here doesn't have a large impact on the in vivo predictions. But this also indicates that the data available aren't sufficient to say that the Km is really different between the tissues evaluated, and a way to possibly reduce the uncertainty among all the parameters is to re-fit the KG, tissue-specific Vmax values, and Km simultaneously to liver, lung, and kidney data; i.e., with a fixed Km.

__However__, I don't suggest going anywhere with that until we get the report from Matt, to resolve the question of the sample volume for the male lung and liver. I recall that it was set at ~ 4 uL in the code in the report Matt sent from the 2nd set of studies (Yuching's code), for simulations of the older data, and while it doesn't show much on the log scale, it makes a difference on linear scale, will impact the optimization I believe.

-Paul





From: Harvey Clewell [mailto:HClewell@ramboll.com]
Sent: Wednesday, October 03, 2018 11:41 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Jerry Campbell <JCampbell@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; cvanlandingham@ramboll.com; Sonja Sax <SSax@ramboll.com>; HIMMELSTEIN, MATTHEW W <Matthew.W.Himmelstein@dupont.com>
Subject: RE: Chloroprene In Vitro model

Sorry Paul, I forgot to mention that these comparisons were run with updated in vitro to in vivo scaling parameters recommended by Miyoung Yoon based on her review of information on protein content that's available now as compared to when Yang et al. (2012) was written. I mentioned that she was doing this when I gave the presentation at EPA and she's now completed it. So you can't compare the results I sent you with the results using the original Yang et al. (2012) scaling. If you'd like we can run the analysis again with the old scaling parameters.

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From: Schlosser, Paul <Schlosser.Paul@epa.gov>
Sent: Wednesday, October 3, 2018 11:31 AM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Jerry Campbell <jcampbell@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; Cynthia Van Landingham <cvanlandingham@ramboll.com>; Sonja Sax <ssax@ramboll.com>; HIMMELSTEIN, MATTHEW W

<Matthew.W.Himmelstein@dupont.com>
Subject: RE: Chloroprene In Vitro model

Harvey,

Something is odd with the results…

First, small thing, the lowest exposure should be 12.8 ppm, not 12.3.

But more significantly, when I run Female_mouse_dose_metric_2.R and _3.R, I get the following:

_2.R

	ppm	AMP	AMPLU	AMPK
1	12.3	1.487844	0.7019859	0.003269034
2	32.0	3.910366	1.1611204	0.008441814
3	80.0	9.739528	1.5521656	0.020442855

_3.R

	ppm	AMP	AMPLU	AMPK
1	12.3	1.473707	0.9477216	0.001063307
2	32.0	3.888278	1.5712141	0.001577733
3	80.0	9.582979	2.1074212	0.001958602

These match almost exactly to the spreadsheet values you sent in August, but differ quite a bit from the ones sent yesterday, "Yang et al. 2012 Table 3 Estimated Metabaolic Point Est. Used" for the female mouse.

Then, running the corresponding male mouse scripts:

_2.R – these numbers are a bit off of what was in the August 3rd spreadsheet. For example, that lists AMPK(80 ppm) = 0.558

	ppm	AMP	AMPLU	AMPK
1	12.3	1.198161	3.797067	0.3334743
2	32.0	3.448833	6.485935	0.4666568
3	80.0	9.298214	8.621115	0.5440947

_3.R – these match the Aug 3 spreadsheet pretty well, just some off by +/- 0.001.

	ppm	AMP	AMPLU	AMPK
1	12.3	1.101225	5.001708	0.3441541
2	32.0	3.286599	8.565978	0.4547611
3	80.0	9.093323	11.280996	0.5152051

But again, quite different from results sent yesterday (left-most set).

It still seems crazy that the total metabolism in the male lung is greater than in the whole liver! But that aside, the baseline tables sent yesterday seem to be off.

-Paul

From: Harvey Clewell [mailto:HClewell@ramboll.com]
Sent: Tuesday, October 02, 2018 5:27 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Jerry Campbell <JCampbell@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; cvanlandingham@ramboll.com; Sonja

Sax <SSax@ramboll.com>; HIMMELSTEIN, MATTHEW W <Matthew.W.Himmelstein@dupont.com>
Subject: RE: Chloroprene In Vitro model

HI Paul

We did check the lung and kidney too. Here's a spreadsheet with all the calculations. The dose metric comparisons I sent you were with all the tissue metabolism parameters re-estimated using the kg estimated from the male mouse liver data. I imagine the effect of kg will be less in the rat than the mouse, but we can check that.

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From: Schlosser, Paul <Schlosser.Paul@epa.gov>
Sent: Tuesday, October 2, 2018 4:06 PM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Jerry Campbell <jcampbell@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; Cynthia Van Landingham <cvanlandingham@ramboll.com>; Sonja Sax <ssax@ramboll.com>; HIMMELSTEIN, MATTHEW W <Matthew.W.Himmelstein@dupont.com>
Subject: RE: Chloroprene In Vitro model

Harvey,

I had thought about doing something like this but was concerned about the identifiability. The Km that you get is *so* low that I suspect the real kg must be higher. When I tested kg = 10x the value from the benzene studies, the Km (eyeball fitting) came out to only ½ the original value, so it's probably really the ratio of the two that we can identify.

That being said, I think what you have here is probably a lower bound on the Km, so if the final model predictions aren't really affected, then the results of this approach can be a bounding sensitivity analysis.

But I think the next question is what happens when this value of kg (and Km) is applied to the mouse lung metabolism data? (And what about the rat?) Perhaps those rates are low enough that it doesn't matter, but that also needs to be determined. Since lung metabolism isn't limited by blood flow, I'd expect a change in the metabolic parameters to have more of an effect on that estimate.

They do still run in vitro metabolism studies in NHEERL, but that's a different lab/center, they have their research plan and budget to consider, etc.

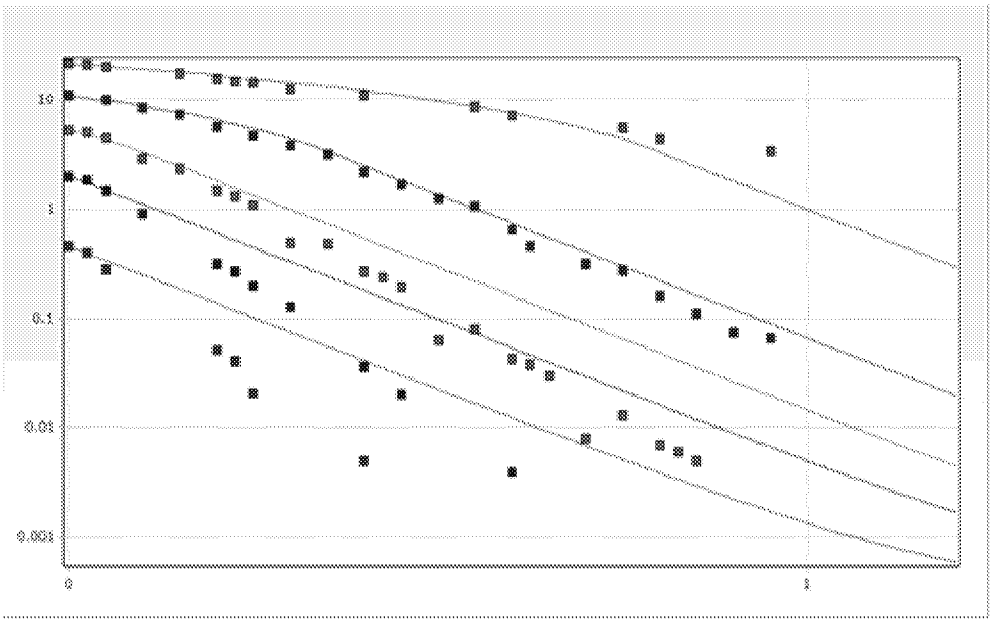
-Paul

From: Harvey Clewell [mailto:HClewell@ramboll.com]
Sent: Tuesday, October 02, 2018 3:27 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Jerry Campbell <JCampbell@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; cvanlandingham@ramboll.com; Sonja Sax <SSax@ramboll.com>; HIMMELSTEIN, MATTHEW W <Matthew.W.Himmelstein@dupont.com>
Subject: RE: Chloroprene In Vitro model

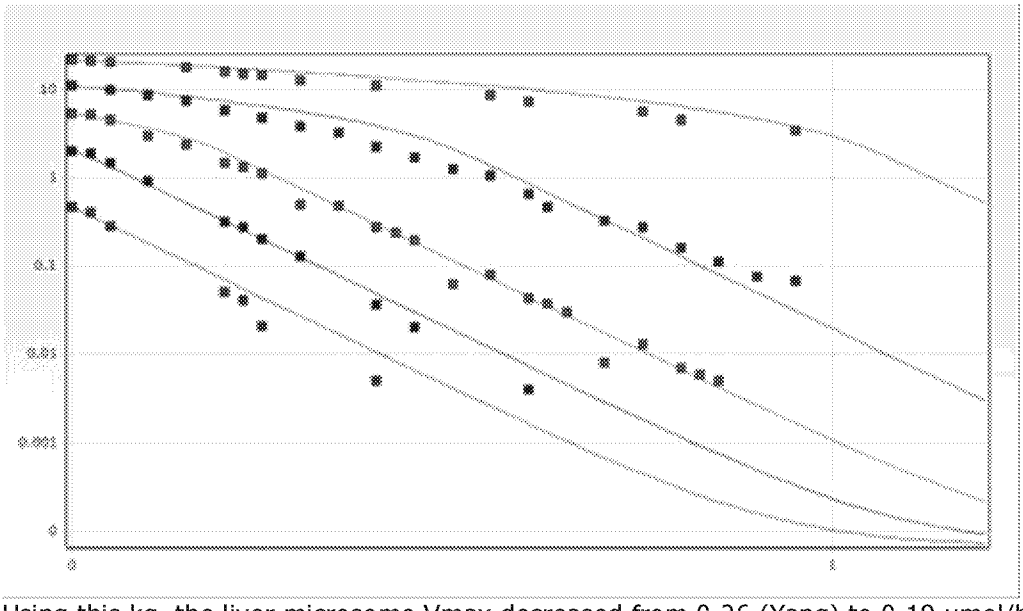
Hi Paul

We've performed an analysis to estimate the potential impact of the use of an equilibrium assumption in the estimation of the in vitro metabolism parameters. Based on this analysis I don't believe it is necessary to conduct new studies to determine a kg for chloroprene. The male mouse liver data are adequate to inform the value of this parameter. Moreover, I'm concerned that such a study would be difficult to perform since the research laboratories that used to conduct such studies with volatiles (WPAFB, CIIT, CSU, Hamner) are no longer available. Does NHEERL still have the capability to perform such a study?

We began, as you did, with the male mouse liver, since that's the strongest data (highest metabolism rates). Scaling the kg from your benzene study (0.434 mL/min= 0.026 L/h) by the ratio of the surface areas in the 4 mL vials you used vs. the 10 mL vials Matt used results in a kg = 0.0636 L/hr and kl = kg/P(liquid/air), which is too low to fit the rate of metabolism at low concentrations.



Next, we used the Nelder-Mead optimization to jointly estimate Vmax, Km, and kg with kl set equal to kg/PC. The resulting estimate of kg was 0.100 L/hr, about 50% higher than the value estimated by scaling your benzene data.



Using this kg, the liver microsome Vmax decreased from 0.26 (Yang) to 0.19 umol/hr/mg protein and the Km decreased from 1.36 to 0.0028 uM. This is again consistent with what you found.

In order to evaluate the potential effect of kg on model predictions we used the kg estimated from the male mouse liver data to re-estimate the metabolism parameters for the liver, lung and kidney in the female mouse. The resulting dose metrics for the female mouse lung are not affected by the use of kg.

Yang et al. 2012 Table 3 Estimated Metabaolic Point Est. Used

Female Mouse Initial Parms			
PPM	AMP	AMPLU	AMPK
12.3	0.60	0.42	0.0015
32	1.60	0.66	0.0022
80	4.04	0.86	0.0027

Benzene KG Adjusted for Vial Surface Area

Female Mouse Initial Parms			
PPM	AMP	AMPLU	AMPK
12.3	0.61	0.42	0.0016
32	1.61	0.67	0.0017
80	4.08	0.86	0.0019

KG Optimized to Male Mouse Liver In Vitro

Female Mouse Initial Parms			
PPM	AMP	AMPLU	AMPK
12.3	0.60	0.41	0.0016
32	1.60	0.66	0.0018
80	4.07	0.86	0.0019

In retrospect, it makes sense that kg doesn't affect the model predictions since the only tissue where metabolism is sufficiently rapid to make kg rate-limiting is the liver, but at the bioassay concentrations liver metabolism is blood-flow limited so decreasing km does not increase the rate of liver metabolism.

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From: Schlosser, Paul <Schlosser.Paul@epa.gov>
Sent: Wednesday, September 19, 2018 2:14 PM
To: HIMMELSTEIN, MATTHEW W <Matthew.W.Himmelstein@dupont.com>; Jerry Campbell <jcampbell@ramboll.com>
Cc: Harvey Clewell <HClewell@ramboll.com>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Thayer, Kris <thayer.kris@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>
Subject: RE: Chloroprene In Vitro model

I'm sending again, including John Vandenberg, Kris Thayer, and Tina Bahadori.

-Paul

From: Schlosser, Paul
Sent: Wednesday, September 19, 2018 1:39 PM
To: 'HIMMELSTEIN, MATTHEW W' <Matthew.W.Himmelstein@dupont.com>; 'Jerry Campbell' <JCampbell@ramboll.com>
Cc: 'Harvey Clewell' <HClewell@ramboll.com>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: Chloroprene In Vitro model

Matt, all,

I'm following up to see how things stand regarding the search for additional data. In a separate note Harvey said there should be a report (IISRP?) for the earlier in vitro studies, which it could help to have. Please send any that you have.

As it stands, we have mostly halted our QA review, as it strongly hinges on the equilibration assumption in the in vitro modeling. The code for the in vitro and in vivo models has checked out, issues resolved, and I think all other parameter discrepancies have been resolved – a few changes but none that should make a really large difference.

I realize it might take some time for files to be retrieved from archives and reviewed, but it's now been a couple of weeks since I provided the written details on what we are seeking. Can you tell us where things stand on your end?

The simulations I've run/provided show that the fits to the low concentration in vitro data depend significantly on the assumption that gas-liquid equilibration is not rate limiting, and the data are consistent with the possibility that it is a factor, requiring a fairly large revision in the estimated Km value(s). As is, my conclusion is that there is uncertainty due to the lack of data on the mass transfer rate, and there isn't an easy way that I can think of (or that we are likely to undertake ourselves) for estimating or bounding that uncertainty. The model results are too uncertain to use, given the data and assumptions.

If data are obtained (from archives or newly developed) that show that mass transfer is a factor, it will then be up to Denka/Ramboll to revise the in vitro parameter estimation accordingly, and propagate that into the in vivo model, before we would continue our QA.

As indicated in previous emails, our QA will also involve comparing model predictions to the nose-only in vivo PK data from 2004: the model should be able to fit with parameters adjusted in a way consistent with the hypothesis that there may be an effect of the exposure system on respiration, but this would not be exposure-concentration-dependent. That will require creating model scripts to run these simulations and compare model outputs to the data. While we are prepared to do that work as part of our QA, provided that the mass transfer data become available, we are not planning to begin that work until those data are available and any necessary revisions of the in vitro modeling have been completed. Alternately, Ramboll colleagues could create the scripts in the meantime, which would speed up the QA.

Sincerely,
-Paul

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~~~~~~~~~~~~~~~~~~~~~~~~

From: Schlosser, Paul
Sent: Wednesday, September 05, 2018 12:06 PM
To: 'HIMMELSTEIN, MATTHEW W' <Matthew.W.Himmelstein@dupont.com>; Jerry Campbell <JCampbell@ramboll.com>
Cc: Harvey Clewell <HClewell@ramboll.com>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: Chloroprene In Vitro model

Matt,

Sorry. I was also wondering at the volume being 1.6 mL bigger than advertised, it seemed like a large discrepancy.

A memo is attached, but here is what I've gotten from looking at the code in the appendix of the report you sent:

- Data to indicate that mass transfer resistance is not significant are still lacking.

- The sample volume (VINJ) for all the CP ***oxidation*** experiments in the 2004 paper should be ~ 400 uL, including male mouse and rat liver and lung data. But the code in the report uses 385.8 uL for male data and exactly 200 uL for male data. Is the higher accuracy for the rodent male and human data supported by some measurements?

- Assuming a similar accuracy, the vial volume (VVIAL) for all experiments described in the 2004 paper should be 0.0120 L. This value should be used for male mouse and rat liver and lung data. (We'll use 0.0116 L for the female mouse and rat data and the kidney data.)

Thanks,
-Paul

Message

From: Schlosser, Paul [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=121CF759D94E4F08AFDE0CEB646E711B-SCHLOSSER, PAUL]
Sent: 6/27/2019 6:14:29 PM
To: Harvey Clewell [HClewell@ramboll.com]
CC: Jerry Campbell [JCampbell@ramboll.com]; Michael Dzierlenga [MDZIERLENGA@ramboll.com]; Robinan Gentry [rgentry@ramboll.com]; mandersen [Personal Matters / Ex. 6]; Thayer, Kris [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3ce4ae3f107749c6815f243260df98c3-Thayer, Kri]; Vandenberg, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=dcae2b98a04540fb8d099f9d4dead690-Vandenberg, John]; Bahadori, Tina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7da7967dcafb4c5bbc39c666fee31ec3-Bahadori, Tina]; Morozov, Viktor [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=03cc9abb639c453fabc2bbb3e4617228-Morozov, Viktor]; Davis, Allen [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=a8ecee8c29c54092b969e9547ea72596-Davis, Allen]; Sasso, Alan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8cb867519abc4dcea88149d12ef3e8e9-Sasso, Alan]; Kapraun, Dustin [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3a53c151b92a472fbfb295ed5df982a7-Kapraun, Du]; Sonja Sax [SSax@ramboll.com]; Ken Mundt [kenneth.mundt@cardno.com]; Miyoung Yoon [Personal Matters / Ex. 6]; Kenyon, Elaina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=0395d5b93f214c8ca49066f498f7d5c9-Kenyon, Elaina]; White, Paul [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=4e179825823c44ebbb07a9704e1e5d16-White, Paul]
Subject: RE: chloroprene -- human lung
Attachments: Yoon_07_extrahepatic-metabolism-cyp2e1-PBPK-VOCs.pdf; Vieira_98.pdf; Vieira_96-European_Journal_of_Biochemistry.pdf

Thanks, Harvey.

The pool size could be bigger, but it is good support that the Lorenz data don't under-estimate human lung activity. … On the other hand, how could I forget?! There is this paper from Miyoung, attached. The ratio shown in table 3 is 0.9%, or 0.009. It is citing

Vieira, I., Pasanen, M., Raunio, H., and Cresteil, T. 1998. Expression of CYP2E1 in human lung and kidney during development and in full-term placenta: A differential methylation of the gene is involved in the regulation process. Pharmacol. Toxicol. 83:183–187, which in turn cites a 1996 article describing the initial tissue collection. Both are also attached, sorry about the rotation of the 1st page of the '98 paper, it's how it is on HERO.

From the '96 paper: Adult liver samples were obtained from donors for kidney transplantation. Donors had no severe chronic pathology and had generally died from a traffic accident. They
had no re-peated drug consumption. No information was available regarding their smoking and drinking habits.

Some plots in the '96 paper indicate 14 donors, others 10 donors.

-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Thursday, June 27, 2019 10:40 AM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen [Personal Matters / Ex. 6]; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon

<Personal Matters / Ex. 6>; Kenyon, Elaina <Kenyon.Elaina@epa.gov>; White, Paul <White.Paul@epa.gov>
Subject: RE: chloroprene -- human lung

Hi Paul

This paper provides relative expression ratios across tissues in the human for a variety of cyps.

From Table 1, the ratio of lung/liver expression for 2e1 is 0.0173/53.8 = 0.00032

Adding 2F1 in the lung (which was not detected in the liver), it becomes (0.0173 + 0.0128)/53.8 = 0.00056

That's about a factor of 3 lower than the lung/liver activity ratio from Lorenz (A1 = 0.00143)

This supports the use of A1 derived from Lorenz to estimate human lung metabolism for 2e1 substrates like chloroprene or methylene chloride as a conservative approach.

With kind regards
Harvey Clewell
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919-452-4279

From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Tuesday, June 25, 2019 5:26 PM
To: Jerry Campbell <JCampbell@ramboll.com>; Harvey Clewell <HClewell@ramboll.com>
Cc: Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen <Personal Matters / Ex. 6> Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon <Personal Matters / Ex. 6>; Kenyon, Elaina <Kenyon.Elaina@epa.gov>; White, Paul <White.Paul@epa.gov>
Subject: chloroprene -- human lung

Harvey, all,

The Lorenz et al. (1984) paper from which the 'A1' for lung:liver metabolism is calculated use 7-ethoxycoumarin as a substrate, which is not a pure 2E1 substrate, but also metabolized by human 1A2, which would not be relevant for CP, and some others.

https://www.ncbi.nlm.nih.gov/pubmed/8573198
https://www.ncbi.nlm.nih.gov/pubmed/16719387

I didn't look thoroughly, but didn't see that Lorenz gave the concentration of 7-EC they used (the 1st reference above indicates that at high concentrations it's more 2E1-specific), and the paper they cite for the method is a review paper. I stopped at that point.

Also, while Lorenz had data on 13 separate human subjects for liver metabolism and 10 for lung (all non-smokers!), they were all having biopsies or surgery for a reason.

No human data set is ideal, but do you know of a 3rd data set for human lung vs. liver activity we could consider, to triangulate the thing, as it were?

-Paul

Message

From: Schlosser, Paul [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=121CF759D94E4F08AFDE0CEB646E711B-SCHLOSSER, PAUL]
Sent: 6/8/2019 1:43:54 PM
To: Harvey Clewell [HClewell@ramboll.com]; Jerry Campbell [JCampbell@ramboll.com]
CC: Michael Dzierlenga [MDZIERLENGA@ramboll.com]; Robinan Gentry [rgentry@ramboll.com]; mandersen Personal Matters / Ex. 6 ayer, Kris [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3ce4ae3f107749c6815f243260df98c3-Thayer, Kri]; Vandenberg, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=dcae2b98a04540fb8d099f9d4dead690-Vandenberg, John]; Bahadori, Tina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7da7967dcafb4c5bbc39c666fee31ec3-Bahadori, Tina]; Morozov, Viktor [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=03cc9abb639c453fabc2bbb3e4617228-Morozov, Viktor]; Davis, Allen [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=a8ecee8c29c54092b969e9547ea72596-Davis, Allen]; Sasso, Alan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8cb867519abc4dcea88149d12ef3e8e9-Sasso, Alan]; Kapraun, Dustin [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3a53c151b92a472fbfb295ed5df982a7-Kapraun, Du]; Sonja Sax [SSax@ramboll.com]; Ken Mundt [kenneth.mundt@cardno.com]; Miyoung Yoon [Personal Matters / Ex. 6]; Kenyon, Elaina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=0395d5b93f214c8ca49066f498f7d5c9-Kenyon, Elaina]
Subject: RE: chloroprene -- in vitro system

P.S. This also folds back on the question of why the male and female mouse metabolic parameters (lung) are so different, when the lung tumor response is not. Throwing up one's hands and saying "that's biology for you" is not satisfying. Maybe the difference is due to a lack of reproducibility of the in vitro kinetics, and the male vs. female experiments being conducted 8 years apart.

I believe there is pharmacodynamic variability, but that much in the same lab, same study (NTP), same species/strain of mouse? I recall that in her postdoc at CIIT, Elaina measured metabolic differences between male and female mice in vivo. I believe those exist. But it would be more convincing if the relative tumor rate, qualitatively, was reflected by the relative metabolic rate, when that rate is supposed to predict mouse-human cancer risk. So I'm trying to assure here that the metabolic constants obtained are not an artifact of a faulty assumption in the model.

-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Friday, June 07, 2019 2:17 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Jerry Campbell <JCampbell@ramboll.com>
Cc: Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen Personal Matters / Ex. 6 ; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon Personal Matters / Ex. 6 m>; Kenyon, Elaina <Kenyon.Elaina@epa.gov>
Subject: RE: chloroprene -- in vitro system

Hi Paul

Not sure why we're including so many folks in this conversation – it's getting pretty arcane.

We definitely appear to be seeing the whole microsome thing from different perspectives. I wouldn't expect there to be is a sufficient mass of microsomes to noticeably affect the partitioning, but according to Miyoung Yoon, who is an expert in this area, non-specific binding to the surface of the microsomes could increase the availability of chloroprene for

metabolism under conditions of mixing (liquid convection). I'll defer to her on that. I think the key uncertainty is what the Reynolds number in the metabolism studies was, which used a shaking/heated auto-sampler, vs. what it was in the rotating water bath in the manual kg study. This, of course, is all somewhat speculative, and I hate it when I come across as a sophist, but I have to say that I am uncomfortable with the use of simple diffusion modeling to predict mass transfer in a convective system.

With kind regards
Harvey Clewell
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From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Friday, June 7, 2019 12:41 PM
To: Jerry Campbell <JCampbell@ramboll.com>; Harvey Clewell <HClewell@ramboll.com>
Cc: Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen <[Personal Matters / Ex. 6]; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon [Personal Matters / Ex. 6]; Kenyon, Elaina <Kenyon.Elaina@epa.gov>
Subject: RE: chloroprene -- in vitro system

Jerry's note and that detail on the experimental procedure just gave me another 'Ah hah!'

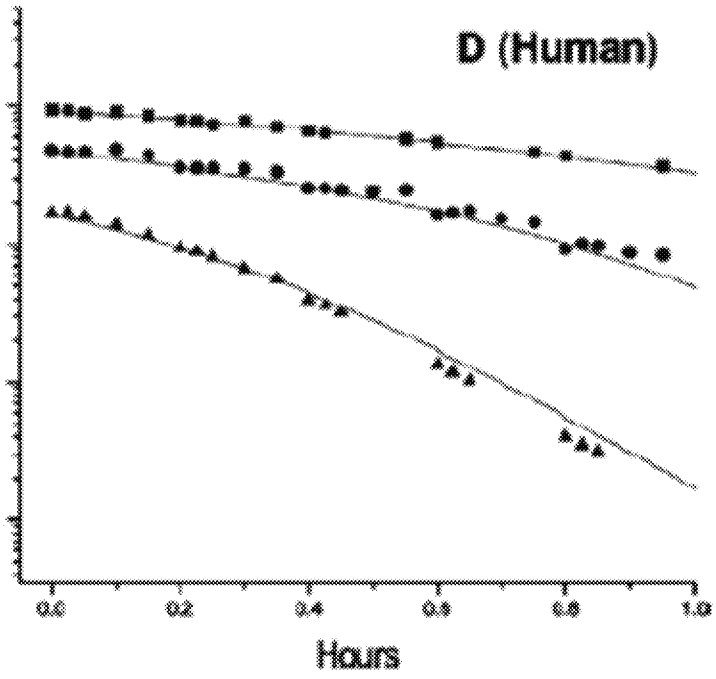
First, adding microsomes after the vials have been given time to equilibrate is based on the assumption that the microsomes don't significantly alter the partitioning. *But*, if it did, that would be evident in the first few minutes of data. You'd see a more rapid decline in the gas phase, like what happens when animals are first placed into a closed-chamber in vivo PK study, as the gas partitions into the animal's tissues. This would be more evident when metabolism is slower, such as with the human in vitro data, plot below. There are data points taken in the first 3 minutes after the microsomes are added, but there is no sign of re-equilibration due to a significant change in gas:liquid partitioning. If that happened, there would be a more rapid decline during that period due to partitioning, after which the rate becomes metabolically limited.

So I've demonstrated that the Kg experiments can be described with metabolic experiments using a consistent set of parameters, by building and testing the model. As the Kg increases, the model will become identical to the original instant-equilibration model, as a diffusion-limited PBPK model becomes indistinguishable from perfusion-limited when the permeability-area (PA) values are high enough. So I'd say it's a more general form, otherwise requires no additional assumptions, besides mass-transfer resistance being a factor.

I'll note that how it impacted the metabolic parameters was not what I had thought, my intuition in that regard failed. This demonstrates why it's important to actually build models and test them if we think they can explain an observation. Our ability to handle the interactions in our heads is limited.

Discussion of how microsomal protein might alter partitioning or mass transfer has so far been just 'talk'. If the data could also be explained by a model based on a change in partitioning (e.g., between the bulk aqueous compartment and a microsomal sub-compartment), that would also be worth evaluating. I leave it to Harvey, Jerry, and colleagues to create the model. However, if that requires proposing that mass transfer of CP bound to microsomal protein is somehow faster than that of free CP in aqueous solution, I'm sure we could find an expert in diffusional transport to serve on the peer review panel and consider the possibility.

-Paul



From: Jerry Campbell <JCampbell@ramboll.com>
Sent: Thursday, June 06, 2019 12:47 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Harvey Clewell <HClewell@ramboll.com>
Cc: Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen **Personal Matters / Ex. 6** yer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon **Personal Matters / Ex. 6** Kenyon, Elaina <Kenyon.Elaina@epa.gov>
Subject: RE: chloroprene -- in vitro system

Paul,

Your use of 1.0 mg/ml for mouse liver in vitro oxidative metabolism of chloroprene is consistent with Matt's 2004 paper. The paper states 0.5 mg/ml for the hydrolysis of 1-CEO (top of right column pg 19 of 2004 paper). The oxidative metabolism in liver was 1.0 mg/mL (last paragraph right column pg 19 of the 2004 paper) and subsequent work in the Yang paper for liver. Higher protein levels - 2 and 3 mg/mL were used in the Yang paper for lung and kidney oxidative metabolism experiments due to lower metabolism but liver was kept at 1.0 mg/mL.

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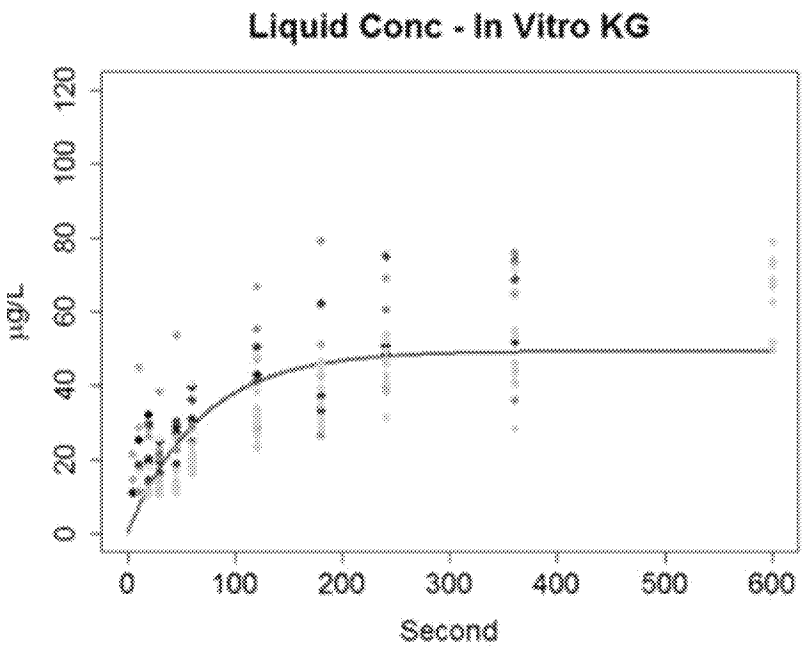
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen Personal Matters / Ex. 6 Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon Personal Matters / Ex. 6 ; Kenyon, Elaina <Kenyon.Elaina@epa.gov>
Subject: RE: chloroprene -- in vitro system

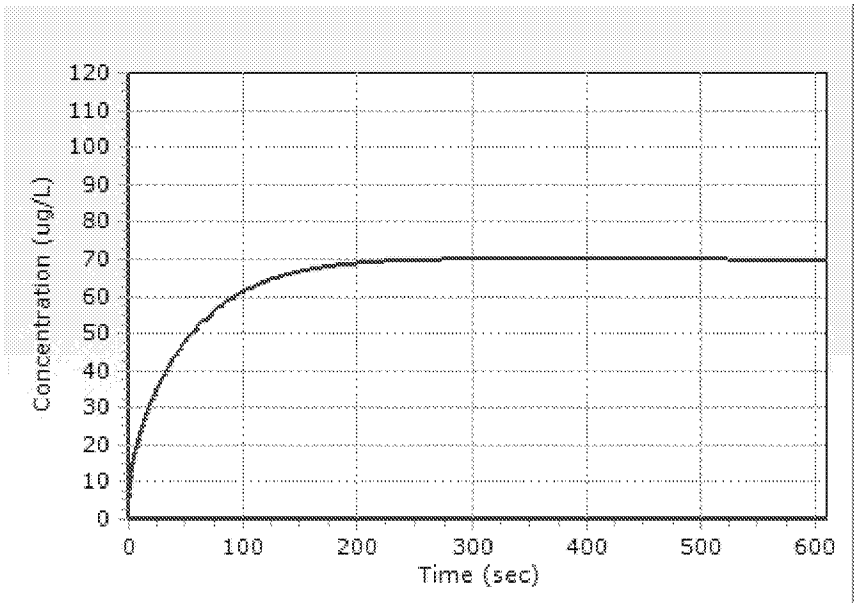
Some preliminary results with a "10-zone" in vitro model. This divides the incubation volume into 10 zones, or layers, though with mixing I don't expect them to be nice, neat layers. Areas near the walls will have less mixing than in the middle.

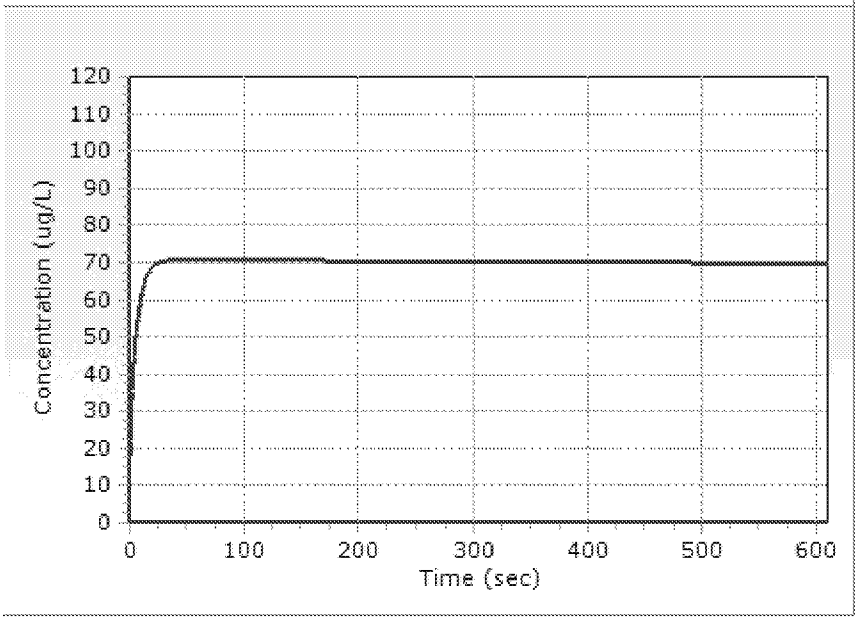
According the supplemental material, sampling in the Kg experiments involved drawing 0.5 ml, or have the total volume of liquid. I presume then that each vial was then sampled only once, and there was no head-space sampling so turned off that term (well, set the fist sample to happen after 10 min). I assumed the rate of loss from the system was the same as measured by Matt Himmelstein. I also assumed that the total volume of the vial was as measured by Matt, 11.65 ml, including the volume in the neck of the bottle. If 0.5 ml of 800 ppm CP are distributed in 10.65 ml of headspace, that works out to an initial concentration of 1.5361 uM by my calculation: 0.5*800/(10.65*24.451). I reduced the initial amount by 20% to match the final steady state in the report plot; we can correct aspect later if need be. The rate constant for transfer between zones is assumed to be 5xKg, under the presumption that Kg represents the resistance between the air and the middle of the vial (5/10 zones), and the rate of transfer from air to the first zone is 10xKg since CP only has to travel through half the zone to its middle.

Given that 0.5/1 ml was being sampled, I assumed that just measured the average concentration in the vial. The plot below shows the result, next to the plot from the report. Other than the difference in the final equilibrium (did I goof the initial concentration calculation? The mass balance checks out), I think it's a fair reproduction, without having to re-estimate Kg.



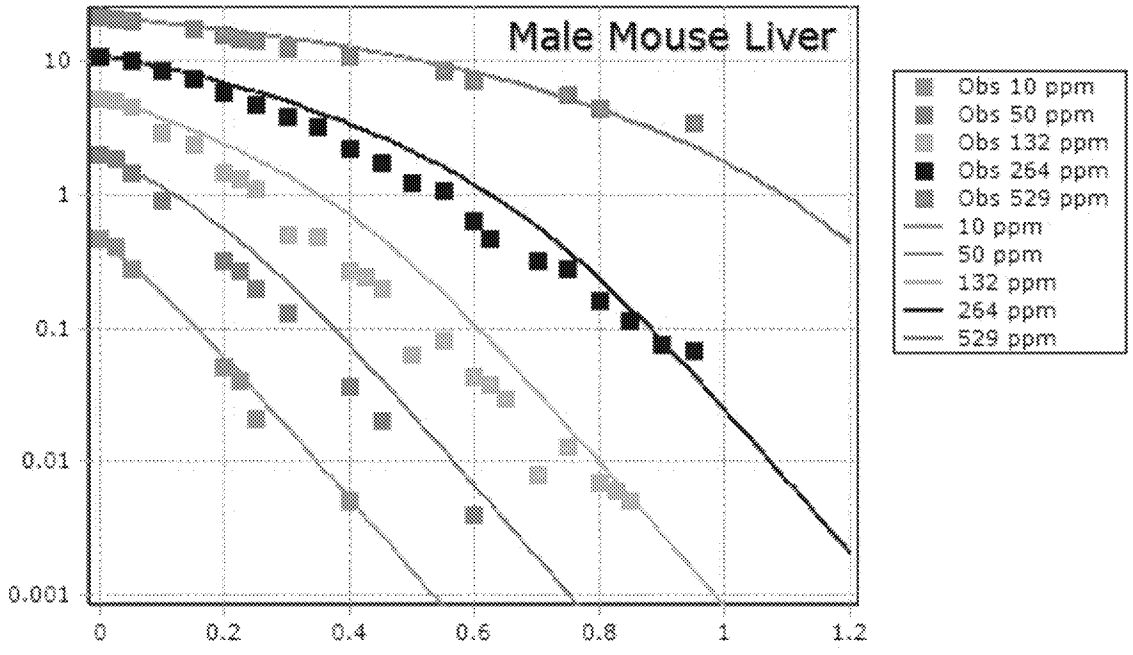
Now I found that using these values of Kg and P as-is did not quite allow me to fit the male mouse liver data. But based on the plot from the report, I thought that using the originally published P (air:water partition coefficient) of 0.69 would be justified, as the final equilibrium is then be in the range of the cluster of points ~ 70 ng/L at 600 seconds (and the higher points at 360 seconds). This was still not enough to allow me to quite fit the metabolic data, but if I tested with Kg = 0.048, double the value estimated, and the Kg listed above, the 'Kg' plot looks like the plot on the left below:

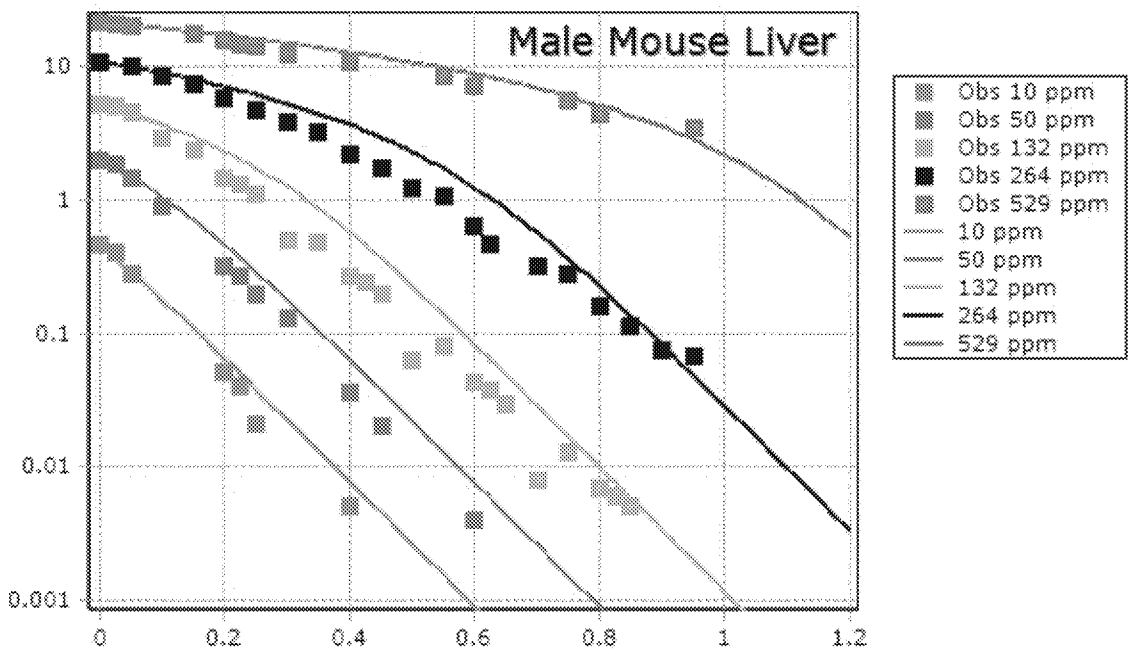




That's definitely faster than the optimized fit from the report, but within the range of the data. In contrast, using KGL=0.45 gives the plot on the right, just above, not consistent with the data. (The slight decline seen after 100 min is due to the system loss term.)

Where does that get us with the metabolism data? Below on the left is after some rough selection of the parameters. Below on the right is with the parameters from the report… setting protein content to 1 mg/ml (Matt's paper says 0.5), which is just doubling the Vmax, and increasing the KG to 1, since this is in zonated model structure. (The report doesn't show a plot like this and I don't feel like switching to the alternate model project just now.) I think these are of comparable quality in fitting the data.





-Paul

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Sent: Wednesday, June 05, 2019 6:34 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; manderser[Personal Matters / Ex. 6]; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon <[Personal Matters / Ex. 6]; Kenyon, Elaina <Kenyon.Elaina@epa.gov>
Subject: RE: chloroprene -- in vitro system

Hi Paul

I would love to be able to get back to doing research to investigate your hypothesis regarding diffusion limitation in the liquid phase. However, even though the experiment you describe may sound simple, performing it correctly would be just as difficult as the original studies conducted by Matt Himmelstein. Unfortunately, there is no longer anyone conducting these kinds of studies. Both John Wambaugh at NCCT and I have tried to identify laboratories with experience in conducting in vitro metabolism studies with volatiles, but we have both been unsuccessful. That is why Denka had to use an environmental contract laboratory to conduct the Kg study.

I have discussed this question with Miyoung Yoon, who is now at FDA, and it was she who suggested that the presence of microsomes in Matt's studies would have greatly increased the availability of chloroprene for metabolism by competing with other sources of non-specific binding. She is the most experienced researcher in the area of *in vitro* metabolism that I know of. I'm afraid this difference in opinion will need to go unresolved, however, not only because the necessary studies are impractical but also because the relevance of any new results to Matt's published studies would be highly uncertain. The difficulty is that Kg is just an empirical parameter that represents the rate of mass transfer under specific experimental conditions. Most importantly, as mixing is increased, the transition from simple diffusion to laminar convection and then to turbulent convection impacts the rate of mass transfer in a nonlinear manner, so extrapolation from one experiment to another would be extremely difficult.

I have also discussed this question with Mel Andersen, and he believes that the published *in vitro* data are completely reliable. He agrees with the approach you suggested for estimating Kg from the male mouse liver metabolism data by fixing Km at the value of 1 uM supported by the literature on cyp2E1 substrates. Re-estimating the metabolism parameters with the estimated Kg results in a 25% decrease in risk compared to using the published values. Values of Kg lower than the value estimated from the metabolism data would reduce risk estimates even further. I just don't see the benefit of performing any additional studies.

With kind regards
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From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Wednesday, June 5, 2019 9:57 AM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; manderse[Personal Matters / Ex. 6]; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon [Personal Matters / Ex. 6]; Kenyon, Elaina <Kenyon.Elaina@epa.gov>
Subject: RE: chloroprene -- in vitro system

I've changed the subject to better reflect the topic.

This morning I realized there's actually a fairly simple experiment that could determine if my hypothesis on diffusion limitation in the liquid phase is correct: run incubations with ½ (or less) of the total incubation mixture, concentrations of microsomes, etc, otherwise the same. (We'd want to have parallel experiments, same lab, same microsomes, etc., with full volume.)

If the system is well mixed, as the current model suggests, then the rate at which chloroprene is removed from the headspace (mass/time) would be reduced by ½, since there's ½ the microsomes doing the work. You'd have to incorporate the change in headspace volume in the calculation (either way).

Alternately, if there is diffusion limitation, with the microsomes near the surface doing the bulk of the metabolism, then the rate of chloroprene removal would not be reduced by ½. A higher fraction of the microsomes would be near the air:liquid interface, so the rate of removal per mg microsome in the system would be higher.

Since the concentration in the incubation solution is effectively calculated by mass balance, this would also lead to an increase in the estimated concentration associated with a given rate of metabolism, I'm pretty sure. The result would then also be an increase in the apparent Km.

Doing these experiments would then evaluate the extent to which mass transport in the liquid phase is limiting in this system, using live/active microsomes, doesn't require any more elaborate analytic methods than already employed.

-Paul

From: Schlosser, Paul
Sent: Tuesday, June 04, 2019 5:14 PM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen Personal Matters / Ex. 6 Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon <Personal Matters / Ex. 6>
Subject: RE: chloroprene -- Bayesian analysis

Harvey, all,

In the Kg experiments, if the sampling of the liquid phase is well into the liquid, away from the air:liquid interface, but mixing is sufficient to keep microsomes evenly distributed, then it's possible for the CP concentration near the surface to be higher, less limited by mass transfer resistance, than in the middle or bottom. If the microsomes near the surface are responsible for the majority of the metabolism, then that could explain the discrepancy between the Kg data/model and the metabolic data.

But that would also mean that the activity of those microsomes was higher than currently estimated… if only 10% of the microsomes (those near the surface) are responsible for the metabolism, the actual Vmax would be 10x higher per mg microsomes, for example. Since the in vivo PK are flow limited, the fits to those data would be the same, if Vmax (in the liver) is actually 10x higher, those in vivo data don't invalidate this hypothesis.

The incubation results would still be linear with microsome content by this explanation, presuming they are well mixed. Using ½ the total microsomes would put that much less near the surface, resulting in a proportional decrease in removal from the gas phase. It's saying that under conditions of high metabolic activity, the assumption of a well-mixed incubation volume is not valid. I think that's more likely than a small fraction of microsomes significantly affecting transport through the entire volume.

Good evening, until tomorrow!

-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Tuesday, June 04, 2019 2:12 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen Personal Matters / Ex. 6 Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonia Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon
Personal Matters / Ex. 6
Subject: RE: chloroprene -- Bayesian analysis

Hi Paul

I do not agree that the apparent discrepancy between the Kg experiments and the metabolism experiments leads to parameter uncertainty. To break the collinearity between Km and Kg, we have followed your suggestion of fixing Km at a value based on the literature for cyp2e1 substrates (1 uM), and have re-estimated Vmax and Kg in the male mouse liver, which shows the highest rates of metabolism. The resulting value of Kg represents the maximum limitation on transport in the in vitro studies that is consistent with the data. It does not demonstrate, however, that there was any significant transport limitation in those studies.

Personally, I have complete confidence in the metabolism data collected by Matt Himmelstein and in the approach he used for its analysis. I see no evidence to support an assumption of transport limitation in his studies. The constants derived in the published analysis are consistent with a large body of work on *in vivo* estimation of kinetic constants for clearance of well-metabolized vapors (i.e., with relatively low Km values). Moreover, assuming that there was a significant limitation on transport in these studies results in Km values that are implausibly low, which in turn results in lower risk estimate compared to use of the published values.

The discussion below of the role of plasma proteins on metabolism is from James Gillette's 1973 paper in the Annals of the NY Academy of Science. He suggested that binding proteins in the plasma can accelerate metabolism by acting as carriers of a drug to the vicinity of the hepatocytes. I believe that microsomal proteins can play a similar role in the *in vitro* studies: in the presence of mixing, non-specific binding of a lipophilic compound to proteins can serve to overcome the transport limitation associated with penetrating the aqueous media. In other words, since chloroprene is lipophilic, diffusion through the aqueous media in the *in vitro* assay would normally be rate-limiting, but if the media is well-mixed and contains microsomal proteins, then non-specific binding of chloroprene to the microsomes could greatly enhance its availability for metabolism.

Unfortunately, investigating this effect in the *in vitro* system would not be at all straightforward, because the microsomal proteins serve both as the site of metabolism and as a source of non-specific binding that competes with the surface of the vial. In fact, I do not believe it is possible to experimentally determine a Kg that would be appropriate in any microsomal metabolism study, because of the dual role that the microsomes play (metabolism and non-specific binding). Denaturing the microsomal proteins in order to eliminate metabolism would also alter their tertiary binding structure.

it is impossible to predict when the dissociation of the drug-protein complex is rate limiting from the association constants alone. It seems probable, however, that the rate of dissociation of the drug-protein complex seldom becomes rate limiting in the metabolism of drugs.

It seems more probable that diffusion of the free drug from the plasma into hepatocytes may be the rate-limiting step in the metabolism of drugs by highly active enzymes in the liver. When this occurs, the bound form of the drug in plasma remains in equilibrium with its free form as the concentration of unbound drug in plasma declines during the passage of the blood through the hepatic sinusoids. However, it is also possible that drug-binding proteins in the cytoplasm of hepatocytes hasten metabolism by maintaining the concentration gradient across the membranes of the hepatocytes and by increasing the amount of drug available to the enzymes located toward the central parts of the cells. Indeed, Y and Z proteins [18–21] are thought to act as carriers of sulfobromophthalein and other anions. But, whether they actually act as carriers or as tissue stores of the anions depends on a number of factors that include the association constants and the concentrations of the proteins as well as on the maximum rate and the K_m values of the drug-metabolizing enzymes and the relative diffusivities of the unbound drug and drug-protein complexes. Because of the complexities of these interrelationships, it is frequently difficult to determine when binding sites in hepatocytes serve as transport mechanisms or as storage mechanisms.

To determine whether plasma proteins act as transport carriers, it is necessary to calculate the apparent clearance on the basis of the concentration of unbound drug in plasma and to compare these apparent clearance values (rate of elimination/concentration of unbound drug) with hepatic blood flow rate. If the apparent clearance exceeds the hepatic blood flow when the drug is known to be eliminated solely by the liver, it may be concluded that the plasma proteins or other components in blood act as transport carriers. When it is not known whether a drug is eliminated solely by the liver, it would be useful to determine whether the clearance value exceeds the cardiac output, because the true clearance can never exceed the cardiac output.

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With kind regards
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Sent: Friday, May 31, 2019 11:41 AM
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Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry

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Subject: RE: chloroprene -- Bayesian analysis

Harvey, all,
(Adding Dustin Kapraun: I'll catch you up later.)

Food for thought for June 12:

Regarding the in vitro analyses, the apparent discrepancy between the "Kg" experiments and the metabolic experiments leads to parameter uncertainty. Kg and the Km values can't be estimated independently from only the metabolic experiments, as you state in the manuscript. But to fully estimate the impact of that uncertainty in subsequent risk estimation, one could potentially use the results of a Bayesian analysis, not just the mean values, but the parameter distributions.

So the first estimation of Kgi and P (from data without microsomes) resulted in a joint distribution of these parameters. Some of those data are consistent with a higher P and Kgi than the mean values – the upper data in Figure B-1. Likewise your review of the literature on Km values effectively provides an informed prior on that parameter. Instead of fixing one or the other of these, formal Bayesian analysis could use those as priors when analyzing the data with active microsomes. I wonder if there are values of Kgi and P consistent with the upper end of the Kg-data (ie, within the uncertainty given those data) that are also consistent with the metabolic data?

I presume the Kg experiments, like the metabolic experiments, involved repeated measures, which needs to be properly accounted for in setting up the likelihood calculation in order to estimate the true uncertainty, full possible range of parameters. The number of independent experiments in Figure B-1 is a lot less than the number of data points, yes? (Since there are clusters of pink points at each time point, it's more than the number of colors used.) The estimated parameter uncertainty estimate would be too low if all the data are treated as independent. Likewise for the metabolic experiments.

If it wasn't done this way (accounting for repeated measures in the likelihood; formal Bayesian sequential parameter estimation), how hard would it be? To unpack this fully, and consider options, we may need to have original data by experimental unit (incubation vial), if it's not already set up that way.

-Paul

From: Schlosser, Paul
Sent: Thursday, May 30, 2019 8:06 AM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: chloroprene

Harvey, all,
(Copying EPA folk)

In the manuscript, you suggest that binding to the microsomal protein, which wasn't present in the Kg-measurement experiments, could have altered the partitioning between air and liquid phases, thereby resulting in the changed mass transfer. If true, this could be explained in the model by changing the water:air partition coefficient. Maybe you can test the hypothesis that way ahead of our meeting, so we know if it's a valid explanation or not. The microsomal concentration was 0.5 mg/ml, ~0.05%, so I don't know that it could alter partitioning too much, but it would be good to know ahead of the 12th if changing the PC alone to any extent could explain the apparent discrepancy.

-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Tuesday, May 14, 2019 4:40 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>
Subject: chloroprene

Hi Paul

Here is the revised manuscript on the chloroprene PBPK model, plus all of the supplemental materials that can be sent via email. The R model and two additional supplemental files (the IISRP report on the in vivo study and the Teklab report on the Kg study) will be transmitted separately, but I don't think you will really need to look at them at this point.

I'm going to be in Netherlands next week for Alina Efremenko's PhD ceremony, so it would be great if we could get together sometime this week to talk about the new analyses documented in the paper. Would that be possible? Jerry and I are free pretty much any time.

With kind regards
Harvey Clewell
PhD, DABT, FATS
Principal Consultant
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919-452-4279

Message

From: Schlosser, Paul [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=121CF759D94E4F08AFDE0CEB646E711B-SCHLOSSER, PAUL]
Sent: 6/18/2019 11:57:57 AM
To: Harvey Clewell [HClewell@ramboll.com]
CC: Michael Dzierlenga [MDZIERLENGA@ramboll.com]; Robinan Gentry [rgentry@ramboll.com]; mandersen Personal Matters / Ex. 6 ayer, Kris [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3ce4ae3f107749c6815f243260df98c3-Thayer, Kri]; Vandenberg, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=dcae2b98a04540fb8d099f9d4dead690-Vandenberg, John]; Bahadori, Tina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7da7967dcafb4c5bbc39c666fee31ec3-Bahadori, Tina]; Morozov, Viktor [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=03cc9abb639c453fabc2bbb3e4617228-Morozov, Viktor]; Davis, Allen [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=a8ecee8c29c54092b969e9547ea72596-Davis, Allen]; Sasso, Alan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8cb867519abc4dcea88149d12ef3e8e9-Sasso, Alan]; Kapraun, Dustin [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3a53c151b92a472fbfb295ed5df982a7-Kapraun, Dus]; Sonja Sax [SSax@ramboll.com]; Ken Mundt [kenneth.mundt@cardno.com]; Miyoung Yoo Personal Matters / Ex. 6 Kenyon, Elaina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=0395d5b93f214c8ca49066f498f7d5c9-Kenyon, Elaina]; Jerry Campbell [JCampbell@ramboll.com]
Subject: RE: chloroprene -- in vitro system

Harvey,

As I am thinking about charge questions for the peer review panel, the result for 'KG' that you got when you fixed the Km at 1 uM might be worth noting for contrast, a kind of sensitivity analysis. It tells us how much larger 'KG' would need to be to achieve that result. I don't think that all the resulting parameter values need to be included (if you've already stripped them from a revised report), but maybe keep in that paragraph or so describing the alternate derivation and where it leads for this parameter.

Also, it really should be denoted 'KGL', since it is a measure of mass transfer through both the gas and liquid phases.

-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Tuesday, June 11, 2019 3:29 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen Personal Matters / Ex. 6 Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon < Personal Matters / Ex. 6 >; Kenyon, Elaina <Kenyon.Elaina@epa.gov>; Jerry Campbell <JCampbell@ramboll.com>
Subject: RE: chloroprene -- in vitro system

I can live with that.

With kind regards
Harvey Clewell
PhD, DABT, FATS
Principal Consultant

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From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Tuesday, June 11, 2019 3:21 PM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen <Personal Matters / Ex. 6>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon <Personal Matters / Ex. 6>; Kenyon, Elaina <Kenyon.Elaina@epa.gov>; Jerry Campbell <JCampbell@ramboll.com>
Subject: RE: chloroprene -- in vitro system

Harvey, Jerry, all,

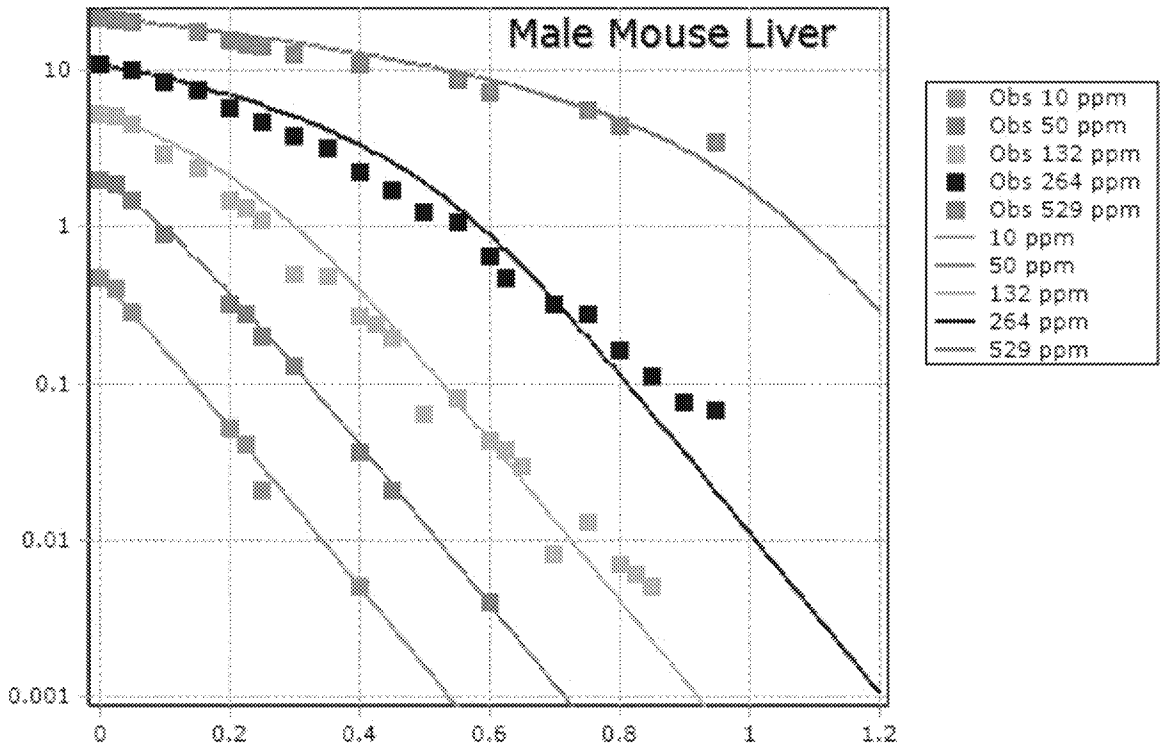
So I am more sure than ever that the addition of 1 mg/ml protein would not make 'KG' any less than in water alone, certainly not an order of magnitude.

But there's something in Matt's methods that seems rather glaring to me now, that I've just confirmed with him by email, given that it's his memory from 15 years ago: apparently he used 500 rpm, while in the KG experiments just run, it was 60. So an independent means of estimating KG would be to use the relative mixing rate.

Setting KG = 0.024*(500/60) = 0.2, I get the plot below with Km = 0.7 uM.

I'm sending this now so you all can let it percholate ahead of tomorrow's discussion. This uses data specific to CP to set parameters, rather than results from other chemicals, including models that have not been QA'd.

-Paul



From: Jerry Campbell <JCampbell@ramboll.com>
Sent: Thursday, June 06, 2019 12:47 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Harvey Clewell <HClewell@ramboll.com>
Cc: Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen <Personal Matters / Ex. 6>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon <Personal Matters / Ex. 6>; Kenyon, Elaina <Kenyon.Elaina@epa.gov>
Subject: RE: chloroprene -- in vitro system

Paul,

Your use of 1.0 mg/ml for mouse liver in vitro oxidative metabolism of chloroprene is consistent with Matt's 2004 paper. The paper states 0.5 mg/ml for the hydrolysis of 1-CEO (top of right column pg 19 of 2004 paper). The oxidative metabolism in liver was 1.0 mg/mL (last paragraph right column pg 19 of the 2004 paper) and subsequent work in the Yang paper for liver. Higher protein levels - 2 and 3 mg/mL were used in the Yang paper for lung and kidney oxidative metabolism experiments due to lower metabolism but liver was kept at 1.0 mg/mL.

Jerry Campbell

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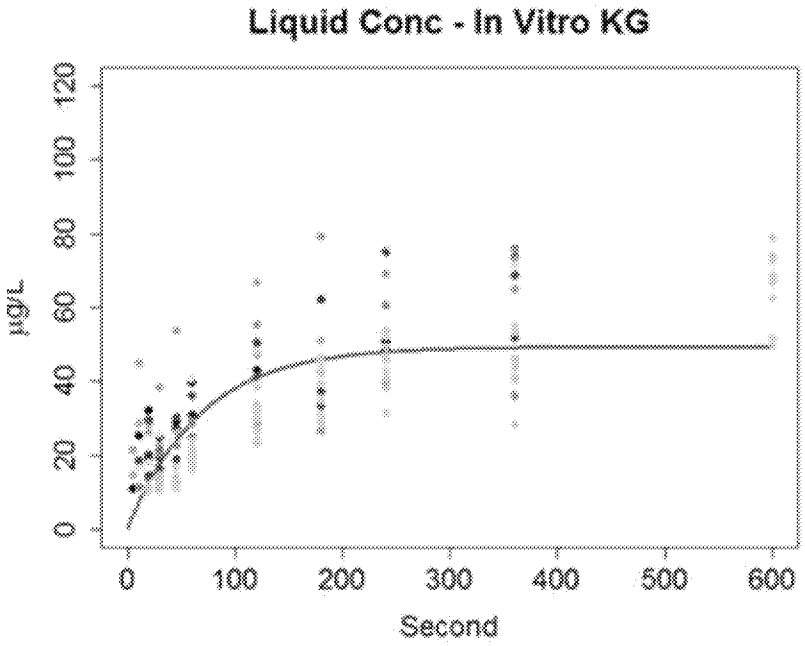
From: Schlosser, Paul <Schlosser.Paul@epa.gov>
Sent: Thursday, June 6, 2019 12:28 PM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; manderser Personal Matters / Ex. 6 ; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon Personal Matters / Ex. 6 ; Kenyon, Elaina <Kenyon.Elaina@epa.gov>
Subject: RE: chloroprene -- in vitro system

Some preliminary results with a "10-zone" in vitro model. This divides the incubation volume into 10 zones, or layers, though with mixing I don't expect them to be nice, neat layers. Areas near the walls will have less mixing than in the middle.

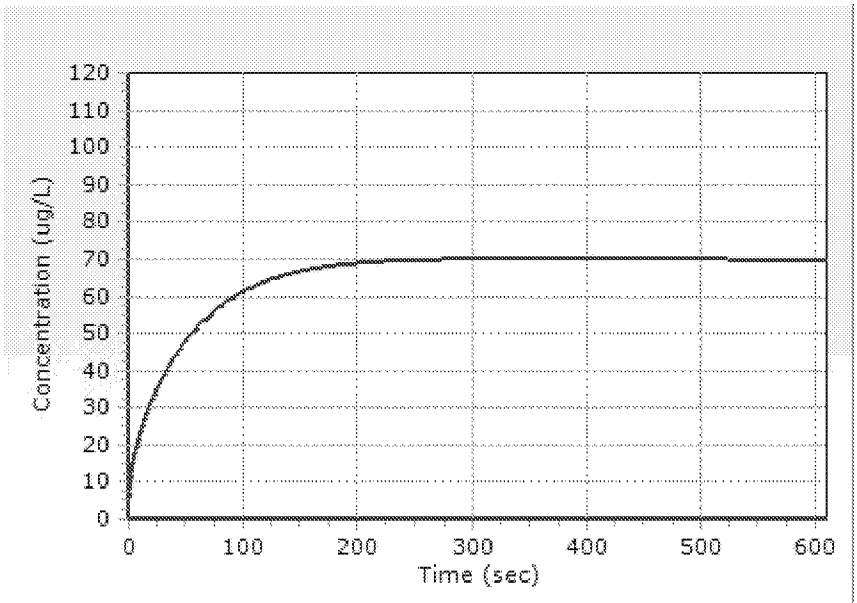
According the supplemental material, sampling in the Kg experiments involved drawing 0.5 ml, or have the total volume of liquid. I presume then that each vial was then sampled only once, and there was no head-space sampling so turned off that term (well, set the fist sample to happen after 10 min). I assumed the rate of loss from the system was the same as measured by Matt Himmelstein. I also assumed that the total volume of the vial was as measured by Matt, 11.65 ml, including the volume in the neck of the bottle. If 0.5 ml of 800 ppm CP are distributed in 10.65 ml of headspace, that works out to an initial concentration of 1.5361 uM by my calculation: 0.5*800/(10.65*24.451). I reduced the initial amount by 20% to match the final steady state in the report plot; we can correct aspect later if need be. The rate constant for transfer between zones is assumed to be 5xKg, under the presumption that Kg

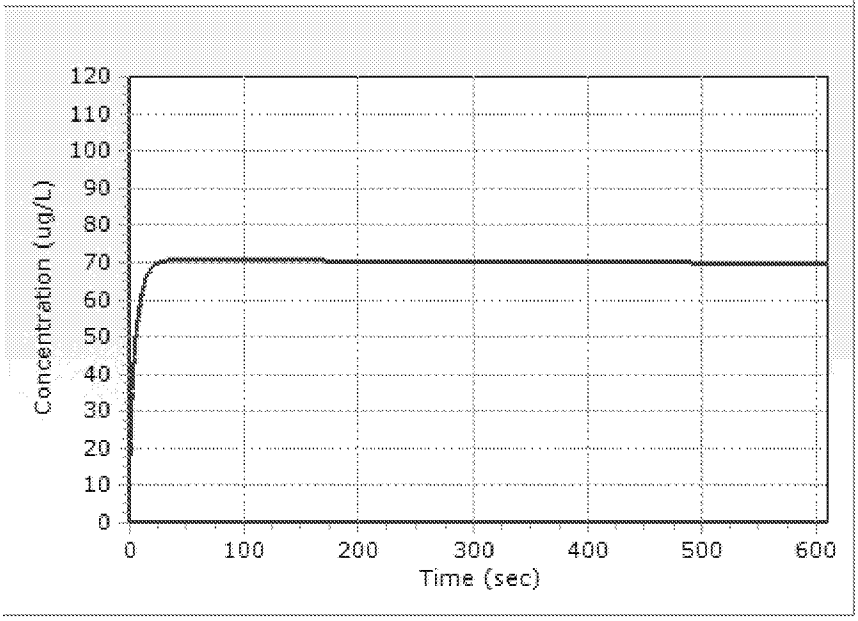
represents the resistance between the air and the middle of the vial (5/10 zones), and the rate of transfer from air to the first zone is 10xKg since CP only has to travel through half the zone to its middle.

Given that 0.5/1 ml was being sampled, I assumed that just measured the average concentration in the vial. The plot below shows the result, next to the plot from the report. Other than the difference in the final equilibrium (did I goof the initial concentration calculation? The mass balance checks out), I think it's a fair reproduction, without having to re-estimate Kg.



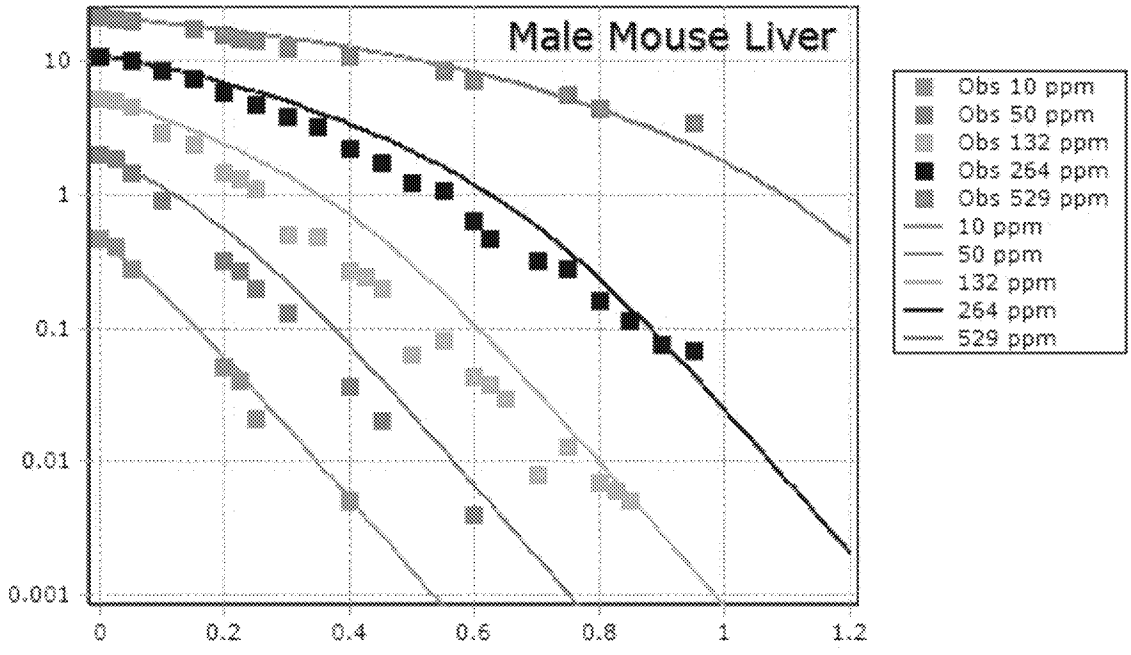
Now I found that using these values of Kg and P as-is did not quite allow me to fit the male mouse liver data. But based on the plot from the report, I thought that using the originally published P (air:water partition coefficient) of 0.69 would be justified, as the final equilibrium is then be in the range of the cluster of points ~ 70 ng/L at 600 seconds (and the higher points at 360 seconds). This was still not enough to allow me to quite fit the metabolic data, but if I tested with Kg = 0.048, double the value estimated, and the Kg listed above, the 'Kg' plot looks like the plot on the left below:

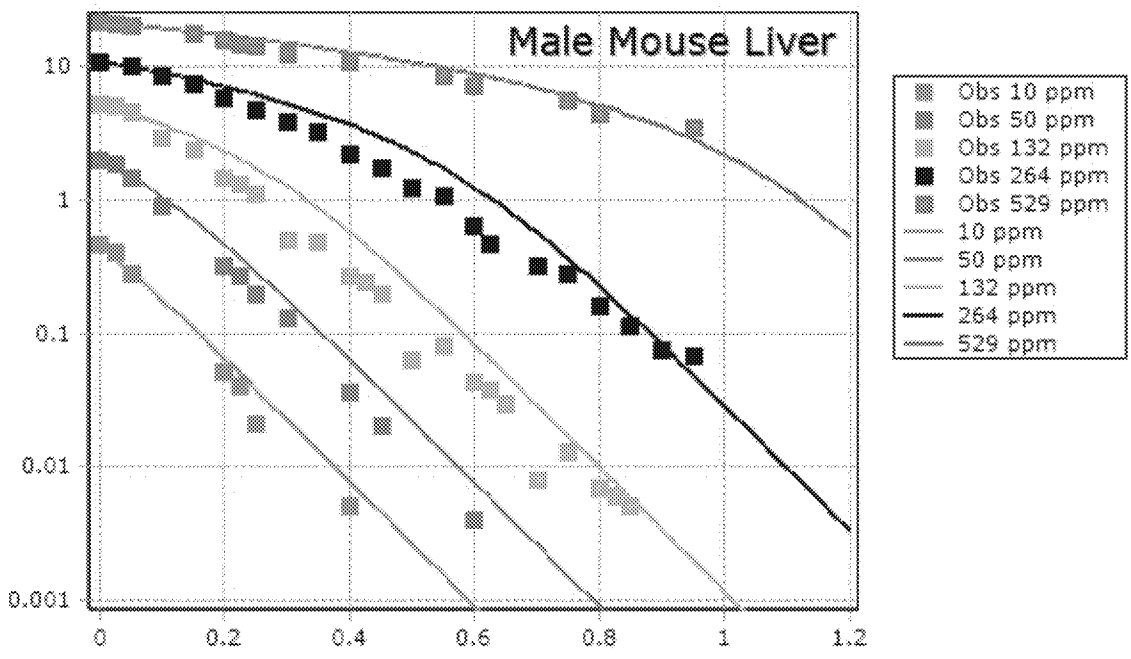




That's definitely faster than the optimized fit from the report, but within the range of the data. In contrast, using KGL=0.45 gives the plot on the right, just above, not consistent with the data. (The slight decline seen after 100 min is due to the system loss term.)

Where does that get us with the metabolism data? Below on the left is after some rough selection of the parameters. Below on the right is with the parameters from the report… setting protein content to 1 mg/ml (Matt's paper says 0.5), which is just doubling the Vmax, and increasing the KG to 1, since this is in zonated model structure. (The report doesn't show a plot like this and I don't feel like switching to the alternate model project just now.) I think these are of comparable quality in fitting the data.





-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Wednesday, June 05, 2019 6:34 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen [Personal Matters / Ex. 6]; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonia Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon [Personal Matters / Ex. 6]; Kenyon, Elaina <Kenyon.Elaina@epa.gov>
Subject: RE: chloroprene -- in vitro system

Hi Paul

I would love to be able to get back to doing research to investigate your hypothesis regarding diffusion limitation in the liquid phase. However, even though the experiment you describe may sound simple, performing it correctly would be just as difficult as the original studies conducted by Matt Himmelstein. Unfortunately, there is no longer anyone conducting these kinds of studies. Both John Wambaugh at NCCT and I have tried to identify laboratories with experience in conducting in vitro metabolism studies with volatiles, but we have both been unsuccessful. That is why Denka had to use an environmental contract laboratory to conduct the Kg study.

I have discussed this question with Miyoung Yoon, who is now at FDA, and it was she who suggested that the presence of microsomes in Matt's studies would have greatly increased the availability of chloroprene for metabolism by competing with other sources of non-specific binding. She is the most experienced researcher in the area of *in vitro* metabolism that I know of. I'm afraid this difference in opinion will need to go unresolved, however, not only because the necessary studies are impractical but also because the relevance of any new results to Matt's published studies would be highly uncertain. The difficulty is that Kg is just an empirical parameter that represents the rate of mass transfer under specific experimental conditions. Most importantly, as mixing is increased, the transition from simple diffusion to laminar convection and then to turbulent convection impacts the rate of mass transfer in a nonlinear manner, so extrapolation from one experiment to another would be extremely difficult.

I have also discussed this question with Mel Andersen, and he believes that the published *in vitro* data are completely reliable. He agrees with the approach you suggested for estimating Kg from the male mouse liver metabolism data by fixing Km at the value of 1 uM supported by the literature on cyp2E1 substrates. Re-estimating the metabolism parameters with the estimated Kg results in a 25% decrease in risk compared to using the published values. Values of Kg lower than the value estimated from the metabolism data would reduce risk estimates even further. I just don't see the benefit of performing any additional studies.

With kind regards
Harvey Clewell
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From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Wednesday, June 5, 2019 9:57 AM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen Personal Matters / Ex. 6 Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <bahadori.tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon Personal Matters / Ex. 6 ; Kenyon, Elaina <Kenyon.Elaina@epa.gov>
Subject: RE: chloroprene -- in vitro system

I've changed the subject to better reflect the topic.

This morning I realized there's actually a fairly simple experiment that could determine if my hypothesis on diffusion limitation in the liquid phase is correct: run incubations with ½ (or less) of the total incubation mixture, concentrations of microsomes, etc, otherwise the same. (We'd want to have parallel experiments, same lab, same microsomes, etc., with full volume.)

If the system is well mixed, as the current model suggests, then the rate at which chloroprene is removed from the headspace (mass/time) would be reduced by ½, since there's ½ the microsomes doing the work. You'd have to incorporate the change in headspace volume in the calculation (either way).

Alternately, if there is diffusion limitation, with the microsomes near the surface doing the bulk of the metabolism, then the rate of chloroprene removal would not be reduced by ½. A higher fraction of the microsomes would be near the air:liquid interface, so the rate of removal per mg microsome in the system would be higher.

Since the concentration in the incubation solution is effectively calculated by mass balance, this would also lead to an increase in the estimated concentration associated with a given rate of metabolism, I'm pretty sure. The result would then also be an increase in the apparent Km.

Doing these experiments would then evaluate the extent to which mass transport in the liquid phase is limiting in this system, using live/active microsomes, doesn't require any more elaborate analytic methods than already employed.

-Paul

From: Schlosser, Paul
Sent: Tuesday, June 04, 2019 5:14 PM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen Personal Matters / Ex. 6 Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon
Personal Matters / Ex. 6
Subject: RE: chloroprene -- Bayesian analysis

Harvey, all,

In the Kg experiments, if the sampling of the liquid phase is well into the liquid, away from the air:liquid interface, but mixing is sufficient to keep microsomes evenly distributed, then it's possible for the CP concentration near the surface to be higher, less limited by mass transfer resistance, than in the middle or bottom. If the microsomes near the surface are responsible for the majority of the metabolism, then that could explain the discrepancy between the Kg data/model and the metabolic data.

But that would also mean that the activity of those microsomes was higher than currently estimated… if only 10% of the microsomes (those near the surface) are responsible for the metabolism, the actual Vmax would be 10x higher per mg microsomes, for example. Since the in vivo PK are flow limited, the fits to those data would be the same, if Vmax (in the liver) is actually 10x higher, those in vivo data don't invalidate this hypothesis.

The incubation results would still be linear with microsome content by this explanation, presuming they are well mixed. Using ½ the total microsomes would put that much less near the surface, resulting in a proportional decrease in removal from the gas phase. It's saying that under conditions of high metabolic activity, the assumption of a well-mixed incubation volume is not valid. I think that's more likely than a small fraction of microsomes significantly affecting transport through the entire volume.

Good evening, until tomorrow!

-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Tuesday, June 04, 2019 2:12 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen Personal Matters / Ex. 6 Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon
Personal Matters / Ex. 6
Subject: RE: chloroprene -- Bayesian analysis

Hi Paul

I do not agree that the apparent discrepancy between the Kg experiments and the metabolism experiments leads to parameter uncertainty. To break the collinearity between Km and Kg, we have followed your suggestion of fixing Km at a value based on the literature for cyp2e1 substrates (1 uM), and have re-estimated Vmax and Kg in the male mouse liver, which shows the highest rates of metabolism. The resulting value of Kg represents the maximum limitation on transport in the in vitro studies that is consistent with the data. It does not demonstrate, however, that there was any significant transport limitation in those studies.

Personally, I have complete confidence in the metabolism data collected by Matt Himmelstein and in the approach he used for its analysis. I see no evidence to support an assumption of transport limitation in his studies. The constants derived in the published analysis are consistent with a large body of work on *in vivo* estimation of kinetic constants for clearance of well-metabolized vapors (i.e., with relatively low Km values). Moreover, assuming that there was a significant limitation on transport in these studies results in Km values that are implausibly low, which in turn results in lower risk estimate compared to use of the published values.

The discussion below of the role of plasma proteins on metabolism is from James Gillette's 1973 paper in the Annals of the NY Academy of Science. He suggested that binding proteins in the plasma can accelerate metabolism by acting as carriers of a drug to the vicinity of the hepatocytes. I believe that microsomal proteins can play a similar role in the *in vitro* studies: in the presence of mixing, non-specific binding of a lipophilic compound to proteins can serve to overcome the transport limitation associated with penetrating the aqueous media. In other words, since chloroprene is lipophilic, diffusion through the aqueous media in the *in vitro* assay would normally be rate-limiting, but if the media is well-mixed and contains microsomal proteins, then non-specific binding of chloroprene to the microsomes could greatly enhance its availability for metabolism.

Unfortunately, investigating this effect in the *in vitro* system would not be at all straightforward, because the microsomal proteins serve both as the site of metabolism and as a source of non-specific binding that competes with the surface of the vial. In fact, I do not believe it is possible to experimentally determine a Kg that would be appropriate in any microsomal metabolism study, because of the dual role that the microsomes play (metabolism and non-specific binding). Denaturing the microsomal proteins in order to eliminate metabolism would also alter their tertiary binding structure.

it is impossible to predict when the dissociation of the drug-protein complex is rate limiting from the association constants alone. It seems probable, however, that the rate of dissociation of the drug-protein complex seldom becomes rate limiting in the metabolism of drugs.

It seems more probable that diffusion of the free drug from the plasma into hepatocytes may be the rate-limiting step in the metabolism of drugs by highly active enzymes in the liver. When this occurs, the bound form of the drug in plasma remains in equilibrium with its free form as the concentration of unbound drug in plasma declines during the passage of the blood through the hepatic sinusoids. However, it is also possible that drug-binding proteins in the cytoplasm of hepatocytes hasten metabolism by maintaining the concentration gradient across the membranes of the hepatocytes and by increasing the amount of drug available to the enzymes located toward the central parts of the cells. Indeed, Y and Z proteins [18–21] are thought to act as carriers of sulfobromophthalein and other anions. But, whether they actually act as carriers or as tissue stores of the anions depends on a number of factors that include the association constants and the concentrations of the proteins as well as on the maximum rate and the K_m values of the drug-metabolizing enzymes and the relative diffusivities of the unbound drug and drug-protein complexes. Because of the complexities of these interrelationships, it is frequently difficult to determine when binding sites in hepatocytes serve as transport mechanisms or as storage mechanisms.

To determine whether plasma proteins act as transport carriers, it is necessary to calculate the apparent clearance on the basis of the concentration of unbound drug in plasma and to compare these apparent clearance values (rate of elimination/concentration of unbound drug) with hepatic blood flow rate. If the apparent clearance exceeds the hepatic blood flow when the drug is known to be eliminated solely by the liver, it may be concluded that the plasma proteins or other components in blood act as transport carriers. When it is not known whether a drug is eliminated solely by the liver, it would be useful to determine whether the clearance value exceeds the cardiac output, because the true clearance can never exceed the cardiac output.

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With kind regards
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From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Friday, May 31, 2019 11:41 AM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry

<rgentry@ramboll.com>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>
Subject: RE: chloroprene -- Bayesian analysis

Harvey, all,
(Adding Dustin Kapraun: I'll catch you up later.)

Food for thought for June 12:

Regarding the in vitro analyses, the apparent discrepancy between the "Kg" experiments and the metabolic experiments leads to parameter uncertainty. Kg and the Km values can't be estimated independently from only the metabolic experiments, as you state in the manuscript. But to fully estimate the impact of that uncertainty in subsequent risk estimation, one could potentially use the results of a Bayesian analysis, not just the mean values, but the parameter distributions.

So the first estimation of Kgi and P (from data without microsomes) resulted in a joint distribution of these parameters. Some of those data are consistent with a higher P and Kgi than the mean values – the upper data in Figure B-1. Likewise your review of the literature on Km values effectively provides an informed prior on that parameter. Instead of fixing one or the other of these, formal Bayesian analysis could use those as priors when analyzing the data with active microsomes. I wonder if there are values of Kgi and P consistent with the upper end of the Kg-data (ie, within the uncertainty given those data) that are also consistent with the metabolic data?

I presume the Kg experiments, like the metabolic experiments, involved repeated measures, which needs to be properly accounted for in setting up the likelihood calculation in order to estimate the true uncertainty, full possible range of parameters. The number of independent experiments in Figure B-1 is a lot less than the number of data points, yes? (Since there are clusters of pink points at each time point, it's more than the number of colors used.) The estimated parameter uncertainty estimate would be too low if all the data are treated as independent. Likewise for the metabolic experiments.

If it wasn't done this way (accounting for repeated measures in the likelihood; formal Bayesian sequential parameter estimation), how hard would it be? To unpack this fully, and consider options, we may need to have original data by experimental unit (incubation vial), if it's not already set up that way.

-Paul

From: Schlosser, Paul
Sent: Thursday, May 30, 2019 8:06 AM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: chloroprene

Harvey, all,
(Copying EPA folk)

In the manuscript, you suggest that binding to the microsomal protein, which wasn't present in the Kg-measurement experiments, could have altered the partitioning between air and liquid phases, thereby resulting in the changed mass transfer. If true, this could be explained in the model by changing the water:air partition coefficient. Maybe you can test the hypothesis that way ahead of our meeting, so we know if it's a valid explanation or not. The microsomal concentration was 0.5 mg/ml, ~0.05%, so I don't know that it could alter partitioning too much, but it would be good to know ahead of the 12th if changing the PC alone to any extent could explain the apparent discrepancy.

-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Tuesday, May 14, 2019 4:40 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>
Subject: chloroprene

Hi Paul

Here is the revised manuscript on the chloroprene PBPK model, plus all of the supplemental materials that can be sent via email. The R model and two additional supplemental files (the IISRP report on the in vivo study and the Teklab report on the Kg study) will be transmitted separately, but I don't think you will really need to look at them at this point.

I'm going to be in Netherlands next week for Alina Efremenko's PhD ceremony, so it would be great if we could get together sometime this week to talk about the new analyses documented in the paper. Would that be possible? Jerry and I are free pretty much any time.

With kind regards
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Message

From: Schlosser, Paul [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=121CF759D94E4F08AFDE0CEB646E711B-SCHLOSSER, PAUL]
Sent: 6/8/2019 11:39:20 AM
To: Harvey Clewell [HClewell@ramboll.com]; Jerry Campbell [JCampbell@ramboll.com]
CC: Michael Dzierlenga [MDZIERLENGA@ramboll.com]; Robinan Gentry [rgentry@ramboll.com]; mandersen [Personal Matters / Ex. 6] Thayer, Kris [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3ce4ae3f107749c6815f243260df98c3-Thayer, Kri]; Vandenberg, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=dcae2b98a04540fb8d099f9d4dead690-Vandenberg, John]; Bahadori, Tina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7da7967dcafb4c5bbc39c666fee31ec3-Bahadori, Tina]; Morozov, Viktor [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=03cc9abb639c453fabc2bbb3e4617228-Morozov, Viktor]; Davis, Allen [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=a8ecee8c29c54092b969e9547ea72596-Davis, Allen]; Sasso, Alan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8cb867519abc4dcea88149d12ef3e8e9-Sasso, Alan]; Kapraun, Dustin [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3a53c151b92a472fbfb295ed5df982a7-Kapraun, Du]; Sonja Sax [SSax@ramboll.com]; Ken Mundt [kenneth.mundt@cardno.com]; Miyoung Yoon [Personal Matters / Ex. 6] Kenyon, Elaina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=0395d5b93f214c8ca49066f498f7d5c9-Kenyon, Elaina]
Subject: RE: chloroprene -- in vitro system

Harvey,

It's important for EPA's process that I keep some of my management chain copied, Allen Davis is the chemical manager, and Alan Sasso, Dustin Kapraun, and Elaina Kenyon are part of the PKWG, assisting with the review.

You are asking me to believe that the in vitro metabolic data collected for the 2004 Himmelstein paper and the 2012 Yang paper are numerically accurate and real measures of the metabolic capacity of mice used in the bioassay described in the 1999 Melnick paper, and that the incubation conditions (degree of mixing) were exactly controlled in the two sets of in vitro experiments such that any resistance to transport in the two was identical (and essentially zero), but somehow the data just collected by Denka is so different that the rate of air-liquid distribution measured therein is completely different… Even though the 'kg' constant obtained for that is within 10% of what I obtained in the early 90's. For metabolic data from different animals taken from a different lab (Haskell) to be applicable to the cancer data from an NTP study, there has to be quantitative similarity despite the difference in animal colonies, genetic drift, housing, age (at which cancer arose vs those sacrificed for tissue microsomes), etc. To me the idea that somehow Matt ran his shakers in a way that yielded essentially perfect mixing on the time-scale of the experiments (that's what one gets with the fitted Kg), when neither I nor the Denka contractors saw that, when this contractor and I got very similar results for the rate of penetration into the medium, is very hard to believe.

I think we need to start by assuming that these new 'kg' data are applicable and comparable to the data collected in 2004 and 2012, that the temperature and degree of mixing were sufficiently similar that they effectively represent the same system. Otherwise it's arguing that these in vitro data aren't reproducible, if we did the metabolic experiments again and didn't mix them exactly the same as Matt did, we could get very different results. I think we need to assume that the system is not so strongly sensitive to the mixing that If the contractor used a degree of agitation similar to what Matt used (based on his methods description), it's not going to change the mixing and transport by a factor of 20.

I'd rather assume the data are reproducible, that transport in the system is similar across these experiments, and that they are simply telling us that the computational model needs some revision, to replace the assumption of perfect mixing.

The 'KGL' term is empirically fitted and hence includes both mixing and diffusion, so to an extent I'm using the term 'diffusion' to represent both. By fitting the model of the system to those 'KG' experiments, we obtain something which accounts for the fact that both mixing and diffusion can only transfer chloroprene from the gas phase into the incubation medium at a finite speed. I used the term 'zones' in describing the distributed version of the model, since I don't imagine there will be strict layers, where penetration is only a function of depth from the surface. Mixing will be higher in the middle than near the walls. But instead of treating the medium as a single, well-mixed compartment, it divides it into 10 sub-compartments representing portions of the volume to which the gas

penetrates more quickly to those to which penetration is slowest. It presumes that movement through the zones is sequential, that chloroprene can't just jump from a region of high concentration to a region of low concentration without passing through regions of intermediate concentration, and the rate of transfer between zones is proportional to the difference in concentration. I think it's a reasonable description of gas penetration into the incubation volume, and it fits the empirical data fairly well. I don't believe it is a less reasonable model of the system than one which assumes perfect and instantaneous mixing throughout the entire medium, when the KG data just collected show that this mixing takes ~ 3-5 min. It's not a perfect model, but it's a better model

Now can the presence of microsomes cause that penetration rate to increase by a factor of 20? Miyoung Yoon may have a lot of expertise with microsomal incubation, but has she been trained in transport phenomena? I have.

First, the computational models of the in vitro system, both 1- and 10-compartment versions, estimate the concentration in the bulk fluid, not the microsomal surface, because they use the total mixture volume (or $1/10^{th}$ the volume per compartment) in the calculation. This is where I am partly challenging you to create an alternate model that splits the volume into aqueous and microsomal environments, given that the microsomes are distributed throughout and separated by aqueous media. That explicitly describes the transfer between the aqueous volume and the microsome surface. But I'll try explain my expectation further.

I do believe that binding to the surface of a microsome can increase the concentration at the enzyme active site locally, relative to the water immediately surrounding the microsome, but over a distance of microns. If that microsome is at the bottom of the reaction vial, the chloroprene still has to get from the surface (air-liquid interface) to the vicinity of the microsome, and to get there it has to travel through the water that is the bulk of the mixture. It is the rate of that movement from the surface across the full depth of the medium that the kg experiments measured, and I do not believe that rate could be increased by the presence of the microsomes. The rate will be proportional to the difference in concentration between sub-regions in the vial (i.e., that in the top of the vial minus that in the bottom), even including mixing. Mixing can only carry the CP at a rate proportional to the concentration in each water drop that is being moved. The concentration in the water drops at the surface cannot be higher than is at equilibrium with the air (since it's all the while being removed by metabolism), and I appreciate you've agreed that the microsomes in the system can't significantly alter that equilibrium ratio, the partitioning. Partitioning between the water and microsome surface on the micro-scale could only alter the concentration in the water by a couple of percent, because the microsome volume is too small in comparison to the surrounding water. And if anything, binding to the surface of microsomes near the air surface would slow down the movement of chloroprene from there to deeper in the vial, since that would reduce the free concentration of CP immediately around the microsome.

Now convection, or mixing, would likely carry microsomes and unbound chloroprene at the same rate – the movement of a drop of water in the system would carry whatever is in that drop at the same speed. But to the extent that the KGL also includes the rate of diffusion (convection will be minimal immediately next to the walls of the vial, due to friction, so diffusion is more significant), the diffusion of microsomes will be slower than that of free chloroprene, because they are bigger. And again, we are only talking about something that is 0.1% of the incubation mixture, so I would expect the impact to be minimal. Hence it seems unlikely at best that non-specific binding to the microsomal surface could result in a 20-fold ***increase*** in the rate of mass transfer between the air and the bulk of the incubation mixture, from the water at the top of the vial to the bottom.

Alternatively, if I use the 10-zone model and a penetration rate constant (KGL) only 2x higher than what fits the new kg data on average, which yields predictions in the range of the kg data, I can fit those metabolic data. Using the KGL estimated with a model that assumes perfect mixing in the medium, you get predictions that are completely inconsistent with those equilibration data.

If you are arguing that the well-mixed model is actually predicting the concentration at the microsome surface, that is possible, but that doesn't jive with the compartment volume (1 mL) being used in the model. You then need to create a model version where the volume is 0.001 mL, not 1 mL, and see what that gives you for parameters. And it changes how the results would need to be interpreted, since the concentration on the microsome surface is the equivalent of the concentration in the liver tissue, not in the venous blood perfusing the tissue, so the PBPK model would need to be changed to use the tissue concentration, not CVtissue, in the metabolic calculations.

-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Friday, June 07, 2019 2:17 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Jerry Campbell <JCampbell@ramboll.com>
Cc: Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen <Personal Matters / Ex. 6> Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon <Personal Matters / Ex. 6>; Kenyon,

Elaina <Kenyon.Elaina@epa.gov>
Subject: RE: chloroprene -- in vitro system

Hi Paul

Not sure why we're including so many folks in this conversation – it's getting pretty arcane.

We definitely appear to be seeing the whole microsome thing from different perspectives. I wouldn't expect there to be is a sufficient mass of microsomes to noticeably affect the partitioning, but according to Miyoung Yoon, who is an expert in this area, non-specific binding to the surface of the microsomes could increase the availability of chloroprene for metabolism under conditions of mixing (liquid convection). I'll defer to her on that. I think the key uncertainty is what the Reynolds number in the metabolism studies was, which used a shaking/heated auto-sampler, vs. what it was in the rotating water bath in the manual kg study. This, of course, is all somewhat speculative, and I hate it when I come across as a sophist, but I have to say that I am uncomfortable with the use of simple diffusion modeling to predict mass transfer in a convective system.

With kind regards
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From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Friday, June 7, 2019 12:41 PM
To: Jerry Campbell <JCampbell@ramboll.com>; Harvey Clewell <HClewell@ramboll.com>
Cc: Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen Personal Matters / Ex. 6 Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon Personal Matters / Ex. 6 ; Kenyon, Elaina <Kenyon.Elaina@epa.gov>
Subject: RE: chloroprene -- in vitro system

Jerry's note and that detail on the experimental procedure just gave me another 'Ah hah!'

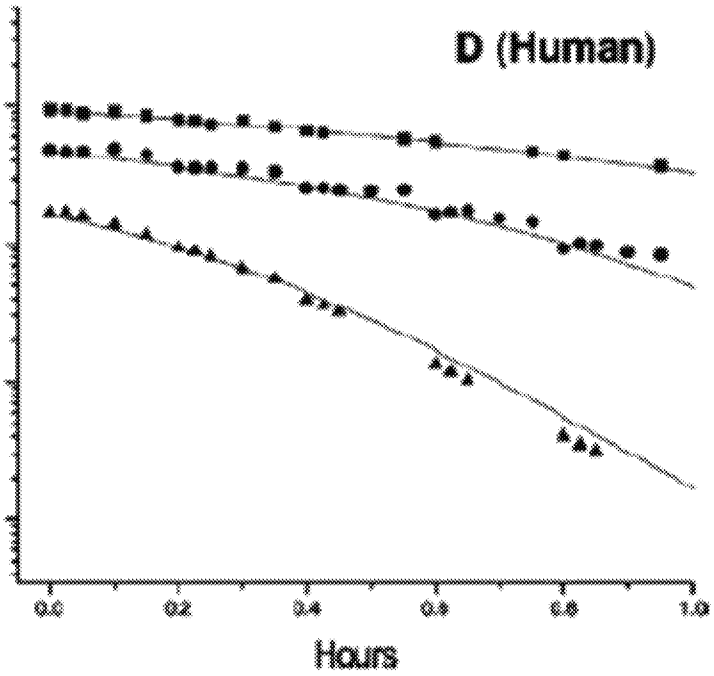
First, adding microsomes after the vials have been given time to equilibrate is based on the assumption that the microsomes don't significantly alter the partitioning. ***But***, if it did, that would be evident in the first few minutes of data. You'd see a more rapid decline in the gas phase, like what happens when animals are first placed into a closed-chamber in vivo PK study, as the gas partitions into the animal's tissues. This would be more evident when metabolism is slower, such as with the human in vitro data, plot below. There are data points taken in the first 3 minutes after the microsomes are added, but there is no sign of re-equilibration due to a significant change in gas:liquid partitioning. If that happened, there would be a more rapid decline during that period due to partitioning, after which the rate becomes metabolically limited.

So I've demonstrated that the Kg experiments can be described with metabolic experiments using a consistent set of parameters, by building and testing the model. As the Kg increases, the model will become identical to the original instant-equilibration model, as a diffusion-limited PBPK model becomes indistinguishable from perfusion-limited when the permeability-area (PA) values are high enough. So I'd say it's a more general form, otherwise requires no additional assumptions, besides mass-transfer resistance being a factor.

I'll note that how it impacted the metabolic parameters was not what I had thought, my intuition in that regard failed. This demonstrates why it's important to actually build models and test them if we think they can explain an observation. Our ability to handle the interactions in our heads is limited.

Discussion of how microsomal protein might alter partitioning or mass transfer has so far been just 'talk'. If the data could also be explained by a model based on a change in partitioning (e.g., between the bulk aqueous compartment and a microsomal sub-compartment), that would also be worth evaluating. I leave it to Harvey, Jerry, and colleagues to create the model. However, if that requires proposing that mass transfer of CP bound to microsomal protein is somehow faster than that of free CP in aqueous solution, I'm sure we could find an expert in diffusional transport to serve on the peer review panel and consider the possibility.

-Paul



From: Jerry Campbell <JCampbell@ramboll.com>
Sent: Thursday, June 06, 2019 12:47 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Harvey Clewell <HClewell@ramboll.com>
Cc: Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen <andersenme@aol.com>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon <yoon.m.work@gmail.com>; Kenyon, Elaina <Kenyon.Elaina@epa.gov>
Subject: RE: chloroprene -- in vitro system

Paul,

Your use of 1.0 mg/ml for mouse liver in vitro oxidative metabolism of chloroprene is consistent with Matt's 2004 paper. The paper states 0.5 mg/ml for the hydrolysis of 1-CEO (top of right column pg 19 of 2004 paper). The oxidative metabolism in liver was 1.0 mg/mL (last paragraph right column pg 19 of the 2004 paper) and subsequent work in the Yang paper for liver. Higher protein levels - 2 and 3 mg/mL were used in the Yang paper for lung and kidney oxidative metabolism experiments due to lower metabolism but liver was kept at 1.0 mg/mL.

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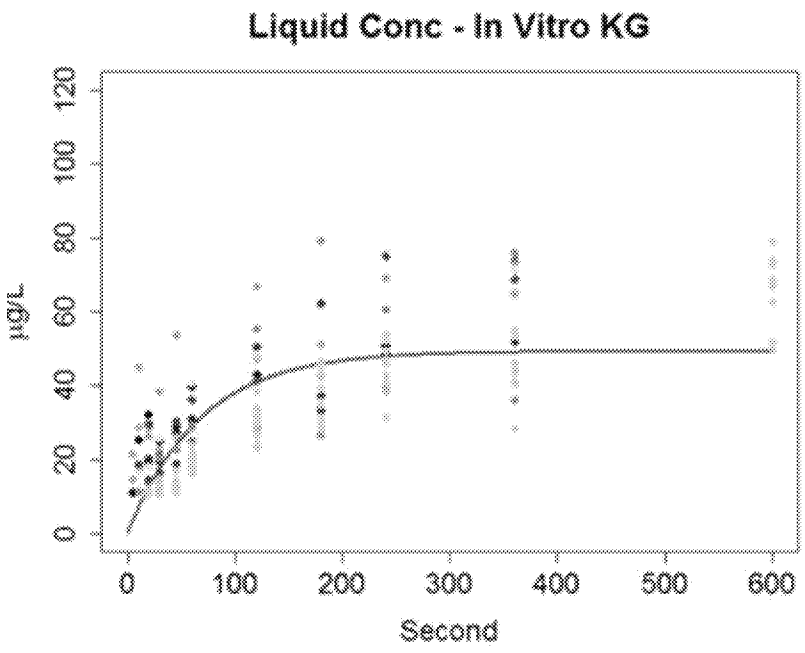
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen <Personal Matters / Ex. 6> Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon <Personal Matters / Ex. 6>; Kenyon, Elaina <Kenyon.Elaina@epa.gov>
Subject: RE: chloroprene -- in vitro system

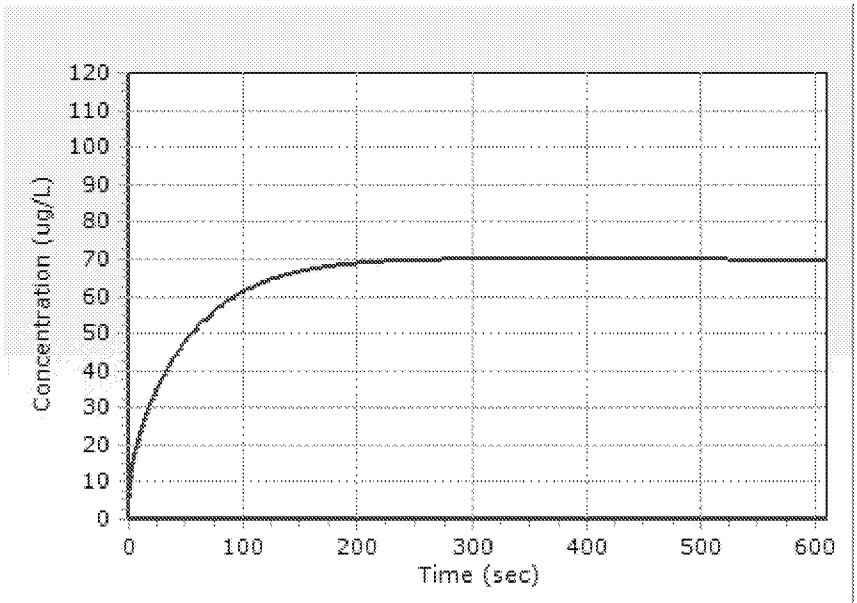
Some preliminary results with a "10-zone" in vitro model. This divides the incubation volume into 10 zones, or layers, though with mixing I don't expect them to be nice, neat layers. Areas near the walls will have less mixing than in the middle.

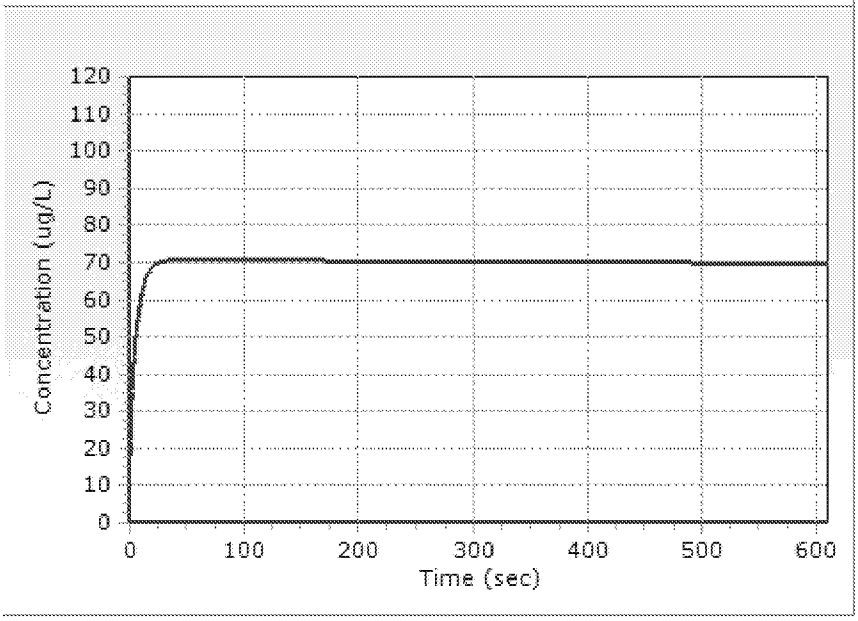
According the supplemental material, sampling in the Kg experiments involved drawing 0.5 ml, or have the total volume of liquid. I presume then that each vial was then sampled only once, and there was no head-space sampling so turned off that term (well, set the fist sample to happen after 10 min). I assumed the rate of loss from the system was the same as measured by Matt Himmelstein. I also assumed that the total volume of the vial was as measured by Matt, 11.65 ml, including the volume in the neck of the bottle. If 0.5 ml of 800 ppm CP are distributed in 10.65 ml of headspace, that works out to an initial concentration of 1.5361 uM by my calculation: 0.5*800/(10.65*24.451). I reduced the initial amount by 20% to match the final steady state in the report plot; we can correct aspect later if need be. The rate constant for transfer between zones is assumed to be 5xKg, under the presumption that Kg represents the resistance between the air and the middle of the vial (5/10 zones), and the rate of transfer from air to the first zone is 10xKg since CP only has to travel through half the zone to its middle.

Given that 0.5/1 ml was being sampled, I assumed that just measured the average concentration in the vial. The plot below shows the result, next to the plot from the report. Other than the difference in the final equilibrium (did I goof the initial concentration calculation? The mass balance checks out), I think it's a fair reproduction, without having to re-estimate Kg.



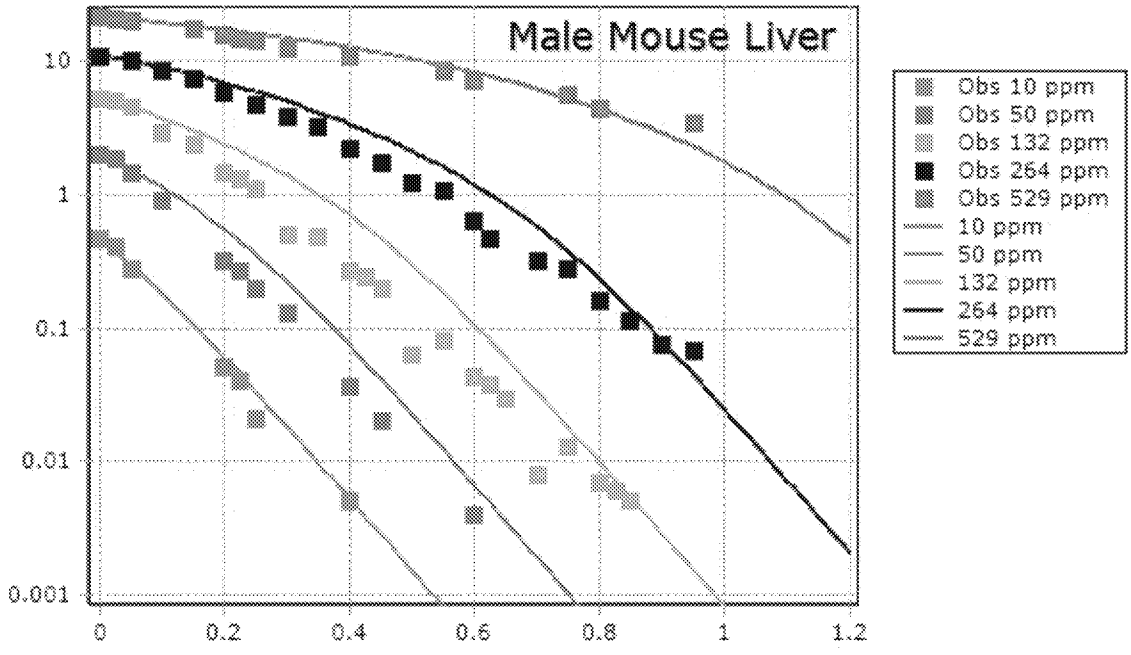
Now I found that using these values of Kg and P as-is did not quite allow me to fit the male mouse liver data. But based on the plot from the report, I thought that using the originally published P (air:water partition coefficient) of 0.69 would be justified, as the final equilibrium is then be in the range of the cluster of points ~ 70 ng/L at 600 seconds (and the higher points at 360 seconds). This was still not enough to allow me to quite fit the metabolic data, but if I tested with Kg = 0.048, double the value estimated, and the Kg listed above, the 'Kg' plot looks like the plot on the left below:

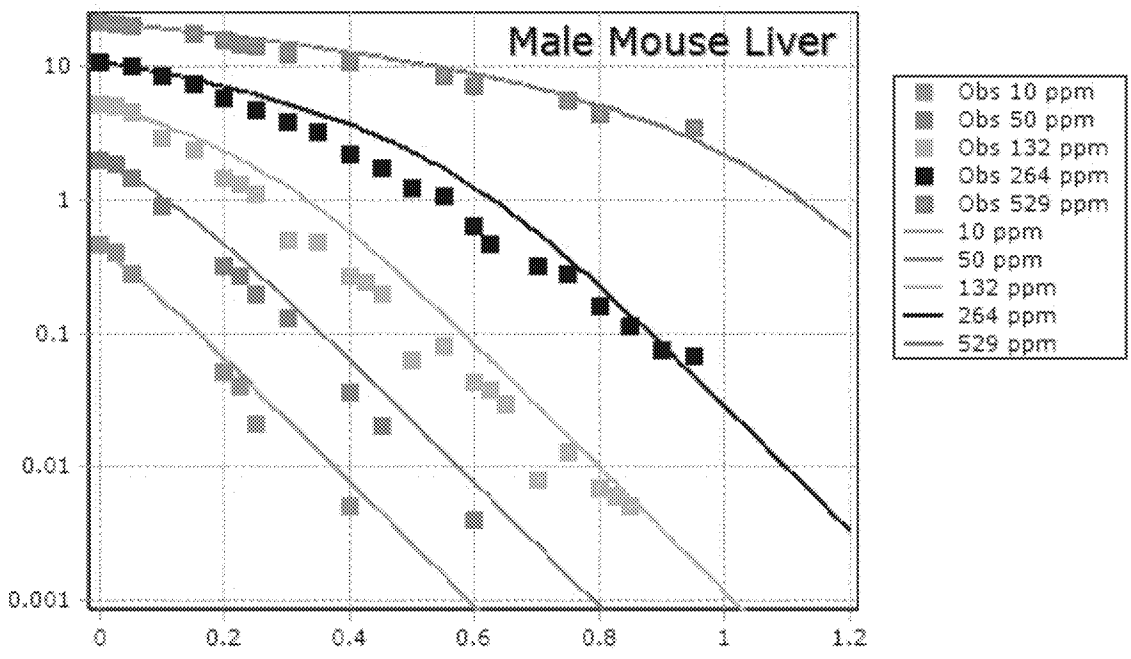




That's definitely faster than the optimized fit from the report, but within the range of the data. In contrast, using KGL=0.45 gives the plot on the right, just above, not consistent with the data. (The slight decline seen after 100 min is due to the system loss term.)

Where does that get us with the metabolism data? Below on the left is after some rough selection of the parameters. Below on the right is with the parameters from the report… setting protein content to 1 mg/ml (Matt's paper says 0.5), which is just doubling the Vmax, and increasing the KG to 1, since this is in zonated model structure. (The report doesn't show a plot like this and I don't feel like switching to the alternate model project just now.) I think these are of comparable quality in fitting the data.





-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Wednesday, June 05, 2019 6:34 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; manderse[Personal Matters / Ex. 6]ayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon <[Personal Matters / Ex. 6]Kenyon, Elaina <Kenyon.Elaina@epa.gov>
Subject: RE: chloroprene -- in vitro system

Hi Paul

I would love to be able to get back to doing research to investigate your hypothesis regarding diffusion limitation in the liquid phase. However, even though the experiment you describe may sound simple, performing it correctly would be just as difficult as the original studies conducted by Matt Himmelstein. Unfortunately, there is no longer anyone conducting these kinds of studies. Both John Wambaugh at NCCT and I have tried to identify laboratories with experience in conducting in vitro metabolism studies with volatiles, but we have both been unsuccessful. That is why Denka had to use an environmental contract laboratory to conduct the Kg study.

I have discussed this question with Miyoung Yoon, who is now at FDA, and it was she who suggested that the presence of microsomes in Matt's studies would have greatly increased the availability of chloroprene for metabolism by competing with other sources of non-specific binding. She is the most experienced researcher in the area of *in vitro* metabolism that I know of. I'm afraid this difference in opinion will need to go unresolved, however, not only because the necessary studies are impractical but also because the relevance of any new results to Matt's published studies would be highly uncertain. The difficulty is that Kg is just an empirical parameter that represents the rate of mass transfer under specific experimental conditions. Most importantly, as mixing is increased, the transition from simple diffusion to laminar convection and then to turbulent convection impacts the rate of mass transfer in a nonlinear manner, so extrapolation from one experiment to another would be extremely difficult.

I have also discussed this question with Mel Andersen, and he believes that the published *in vitro* data are completely reliable. He agrees with the approach you suggested for estimating Kg from the male mouse liver metabolism data by fixing Km at the value of 1 uM supported by the literature on cyp2E1 substrates. Re-estimating the metabolism parameters with the estimated Kg results in a 25% decrease in risk compared to using the published values. Values of Kg lower than the value estimated from the metabolism data would reduce risk estimates even further. I just don't see the benefit of performing any additional studies.

With kind regards
Harvey Clewell
PhD, DABT, FATS
Principal Consultant
Ramboll Environment and Health Consulting
Research Triangle Park, NC 27709 USA
hclewell@ramboll.com
919-452-4279

From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Wednesday, June 5, 2019 9:57 AM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; manderser[Personal Matters / Ex. 6]; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon [Personal Matters / Ex. 6]; Kenyon, Elaina <Kenyon.Elaina@epa.gov>
Subject: RE: chloroprene -- in vitro system

I've changed the subject to better reflect the topic.

This morning I realized there's actually a fairly simple experiment that could determine if my hypothesis on diffusion limitation in the liquid phase is correct: run incubations with ½ (or less) of the total incubation mixture, concentrations of microsomes, etc, otherwise the same. (We'd want to have parallel experiments, same lab, same microsomes, etc., with full volume.)

If the system is well mixed, as the current model suggests, then the rate at which chloroprene is removed from the headspace (mass/time) would be reduced by ½, since there's ½ the microsomes doing the work. You'd have to incorporate the change in headspace volume in the calculation (either way).

Alternately, if there is diffusion limitation, with the microsomes near the surface doing the bulk of the metabolism, then the rate of chloroprene removal would not be reduced by ½. A higher fraction of the microsomes would be near the air:liquid interface, so the rate of removal per mg microsome in the system would be higher.

Since the concentration in the incubation solution is effectively calculated by mass balance, this would also lead to an increase in the estimated concentration associated with a given rate of metabolism, I'm pretty sure. The result would then also be an increase in the apparent Km.

Doing these experiments would then evaluate the extent to which mass transport in the liquid phase is limiting in this system, using live/active microsomes, doesn't require any more elaborate analytic methods than already employed.

-Paul

From: Schlosser, Paul
Sent: Tuesday, June 04, 2019 5:14 PM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen Personal Matters / Ex. 6 ; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon
Personal Matters / Ex. 6
Subject: RE: chloroprene -- Bayesian analysis

Harvey, all,

In the Kg experiments, if the sampling of the liquid phase is well into the liquid, away from the air:liquid interface, but mixing is sufficient to keep microsomes evenly distributed, then it's possible for the CP concentration near the surface to be higher, less limited by mass transfer resistance, than in the middle or bottom. If the microsomes near the surface are responsible for the majority of the metabolism, then that could explain the discrepancy between the Kg data/model and the metabolic data.

But that would also mean that the activity of those microsomes was higher than currently estimated… if only 10% of the microsomes (those near the surface) are responsible for the metabolism, the actual Vmax would be 10x higher per mg microsomes, for example. Since the in vivo PK are flow limited, the fits to those data would be the same, if Vmax (in the liver) is actually 10x higher, those in vivo data don't invalidate this hypothesis.

The incubation results would still be linear with microsome content by this explanation, presuming they are well mixed. Using ½ the total microsomes would put that much less near the surface, resulting in a proportional decrease in removal from the gas phase. It's saying that under conditions of high metabolic activity, the assumption of a well-mixed incubation volume is not valid. I think that's more likely than a small fraction of microsomes significantly affecting transport through the entire volume.

Good evening, until tomorrow!

-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Tuesday, June 04, 2019 2:12 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; mandersen Personal Matters / Ex. 6 ; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon
Personal Matters / Ex. 6
Subject: RE: chloroprene -- Bayesian analysis

Hi Paul

I do not agree that the apparent discrepancy between the Kg experiments and the metabolism experiments leads to parameter uncertainty. To break the collinearity between Km and Kg, we have followed your suggestion of fixing Km at a value based on the literature for cyp2e1 substrates (1 uM), and have re-estimated Vmax and Kg in the male mouse liver, which shows the highest rates of metabolism. The resulting value of Kg represents the maximum limitation on transport in the in vitro studies that is consistent with the data. It does not demonstrate, however, that there was any significant transport limitation in those studies.

Personally, I have complete confidence in the metabolism data collected by Matt Himmelstein and in the approach he used for its analysis. I see no evidence to support an assumption of transport limitation in his studies. The constants derived in the published analysis are consistent with a large body of work on *in vivo* estimation of kinetic constants for clearance of well-metabolized vapors (i.e., with relatively low Km values). Moreover, assuming that there was a significant limitation on transport in these studies results in Km values that are implausibly low, which in turn results in lower risk estimate compared to use of the published values.

The discussion below of the role of plasma proteins on metabolism is from James Gillette's 1973 paper in the Annals of the NY Academy of Science. He suggested that binding proteins in the plasma can accelerate metabolism by acting as carriers of a drug to the vicinity of the hepatocytes. I believe that microsomal proteins can play a similar role in the *in vitro* studies: in the presence of mixing, non-specific binding of a lipophilic compound to proteins can serve to overcome the transport limitation associated with penetrating the aqueous media. In other words, since chloroprene is lipophilic, diffusion through the aqueous media in the *in vitro* assay would normally be rate-limiting, but if the media is well-mixed and contains microsomal proteins, then non-specific binding of chloroprene to the microsomes could greatly enhance its availability for metabolism.

Unfortunately, investigating this effect in the *in vitro* system would not be at all straightforward, because the microsomal proteins serve both as the site of metabolism and as a source of non-specific binding that competes with the surface of the vial. In fact, I do not believe it is possible to experimentally determine a Kg that would be appropriate in any microsomal metabolism study, because of the dual role that the microsomes play (metabolism and non-specific binding). Denaturing the microsomal proteins in order to eliminate metabolism would also alter their tertiary binding structure.

it is impossible to predict when the dissociation of the drug-protein complex is rate limiting from the association constants alone. It seems probable, however, that the rate of dissociation of the drug-protein complex seldom becomes rate limiting in the metabolism of drugs.

It seems more probable that diffusion of the free drug from the plasma into hepatocytes may be the rate-limiting step in the metabolism of drugs by highly active enzymes in the liver. When this occurs, the bound form of the drug in plasma remains in equilibrium with its free form as the concentration of unbound drug in plasma declines during the passage of the blood through the hepatic sinusoids. However, it is also possible that drug-binding proteins in the cytoplasm of hepatocytes hasten metabolism by maintaining the concentration gradient across the membranes of the hepatocytes and by increasing the amount of drug available to the enzymes located toward the central parts of the cells. Indeed, Y and Z proteins [18–21] are thought to act as carriers of sulfobromophthalein and other anions. But, whether they actually act as carriers or as tissue stores of the anions depends on a number of factors that include the association constants and the concentrations of the proteins as well as on the maximum rate and the K_m values of the drug-metabolizing enzymes and the relative diffusivities of the unbound drug and drug-protein complexes. Because of the complexities of these interrelationships, it is frequently difficult to determine when binding sites in hepatocytes serve as transport mechanisms or as storage mechanisms.

To determine whether plasma proteins act as transport carriers, it is necessary to calculate the apparent clearance on the basis of the concentration of unbound drug in plasma and to compare these apparent clearance values (rate of elimination/concentration of unbound drug) with hepatic blood flow rate. If the apparent clearance exceeds the hepatic blood flow when the drug is known to be eliminated solely by the liver, it may be concluded that the plasma proteins or other components in blood act as transport carriers. When it is not known whether a drug is eliminated solely by the liver, it would be useful to determine whether the clearance value exceeds the cardiac output, because the true clearance can never exceed the cardiac output.

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With kind regards
Harvey Clewell
PhD, DABT, FATS
Principal Consultant
Ramboll Environment and Health Consulting
Research Triangle Park, NC 27709 USA
hclewell@ramboll.com
919-452-4279

From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Friday, May 31, 2019 11:41 AM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry

<rgentry@ramboll.com>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>
Subject: RE: chloroprene -- Bayesian analysis

Harvey, all,
(Adding Dustin Kapraun: I'll catch you up later.)

Food for thought for June 12:

Regarding the in vitro analyses, the apparent discrepancy between the "Kg" experiments and the metabolic experiments leads to parameter uncertainty. Kg and the Km values can't be estimated independently from only the metabolic experiments, as you state in the manuscript. But to fully estimate the impact of that uncertainty in subsequent risk estimation, one could potentially use the results of a Bayesian analysis, not just the mean values, but the parameter distributions.

So the first estimation of Kgi and P (from data without microsomes) resulted in a joint distribution of these parameters. Some of those data are consistent with a higher P and Kgi than the mean values – the upper data in Figure B-1. Likewise your review of the literature on Km values effectively provides an informed prior on that parameter. Instead of fixing one or the other of these, formal Bayesian analysis could use those as priors when analyzing the data with active microsomes. I wonder if there are values of Kgi and P consistent with the upper end of the Kg-data (ie, within the uncertainty given those data) that are also consistent with the metabolic data?

I presume the Kg experiments, like the metabolic experiments, involved repeated measures, which needs to be properly accounted for in setting up the likelihood calculation in order to estimate the true uncertainty, full possible range of parameters. The number of independent experiments in Figure B-1 is a lot less than the number of data points, yes? (Since there are clusters of pink points at each time point, it's more than the number of colors used.) The estimated parameter uncertainty estimate would be too low if all the data are treated as independent. Likewise for the metabolic experiments.

If it wasn't done this way (accounting for repeated measures in the likelihood; formal Bayesian sequential parameter estimation), how hard would it be? To unpack this fully, and consider options, we may need to have original data by experimental unit (incubation vial), if it's not already set up that way.

-Paul

From: Schlosser, Paul
Sent: Thursday, May 30, 2019 8:06 AM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: chloroprene

Harvey, all,
(Copying EPA folk)

In the manuscript, you suggest that binding to the microsomal protein, which wasn't present in the Kg-measurement experiments, could have altered the partitioning between air and liquid phases, thereby resulting in the changed mass transfer. If true, this could be explained in the model by changing the water:air partition coefficient. Maybe you can test the hypothesis that way ahead of our meeting, so we know if it's a valid explanation or not. The microsomal concentration was 0.5 mg/ml, ~ 0.05%, so I don't know that it could alter partitioning too much, but it would be good to know ahead of the 12th if changing the PC alone to any extent could explain the apparent discrepancy.

-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Tuesday, May 14, 2019 4:40 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>
Subject: chloroprene

Hi Paul

Here is the revised manuscript on the chloroprene PBPK model, plus all of the supplemental materials that can be sent via email. The R model and two additional supplemental files (the IISRP report on the in vivo study and the Teklab report on the Kg study) will be transmitted separately, but I don't think you will really need to look at them at this point.

I'm going to be in Netherlands next week for Alina Efremenko's PhD ceremony, so it would be great if we could get together sometime this week to talk about the new analyses documented in the paper. Would that be possible? Jerry and I are free pretty much any time.

With kind regards
Harvey Clewell
PhD, DABT, FATS
Principal Consultant
Ramboll Environment and Health Consulting
Research Triangle Park, NC 27709 USA
hclewell@ramboll.com
919-452-4279

Message

From: Schlosser, Paul [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=121CF759D94E4F08AFDE0CEB646E711B-SCHLOSSER, PAUL]
Sent: 4/19/2019 7:51:44 PM
To: Jerry Campbell [JCampbell@ramboll.com]; Harvey Clewell [HClewell@ramboll.com]
Subject: RE: what else?

The fact that the kg data and the metabolism data are inconsistent is still troubling. And you all might not have realized this, but until now we have not used *any* human PBPK model where there aren't any human in vivo PK data to evaluate/calibrate the model. If it can be shown that at least the IVIVE is predictive for what animal in vivo PK data there are, that would help.

-Paul

From: Jerry Campbell <JCampbell@ramboll.com>
Sent: Tuesday, April 16, 2019 1:25 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Harvey Clewell <HClewell@ramboll.com>
Subject: RE: what else?

Front door will work.

Jerry

From: Schlosser, Paul [Schlosser.Paul@epa.gov]
Sent: 16 April 2019 19:22
To: Jerry Campbell; Harvey Clewell
Subject: RE: what else?

Got it, will see you then. Front entrance, right?

From: Jerry Campbell <JCampbell@ramboll.com>
Sent: Tuesday, April 16, 2019 1:08 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>; Harvey Clewell <HClewell@ramboll.com>
Subject: RE: what else?

Call my cell, Personal Matters / Ex. 6 when you get to the building and I'll let you in.

Jerry

From: Schlosser, Paul [Schlosser.Paul@epa.gov]
Sent: 16 April 2019 18:52
To: Harvey Clewell
Cc: Jerry Campbell
Subject: RE: what else?

On my calendar. I'll just come there.

-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Tuesday, April 16, 2019 12:35 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Jerry Campbell <JCampbell@ramboll.com>
Subject: RE: what else?

1:00?

With kind regards
Harvey Clewell
PhD, DABT, FATS
Principal Consultant
Ramboll Environment and Health Consulting
Research Triangle Park, NC 27709 USA
hclewell@ramboll.com
919-452-4279

From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Tuesday, April 16, 2019 12:32 PM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>
Subject: RE: what else?

So what time Friday?

-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Tuesday, April 16, 2019 12:21 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Jerry Campbell <JCampbell@ramboll.com>
Subject: RE: what else?

Hi Paul

Jerry says Friday afternoon would be best, but if not then early Thursday afternoon.

With kind regards
Harvey Clewell
PhD, DABT, FATS
Principal Consultant
Ramboll Environment and Health Consulting
Research Triangle Park, NC 27709 USA
hclewell@ramboll.com
919-452-4279

From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Tuesday, April 16, 2019 8:52 AM
To: Harvey Clewell <HClewell@ramboll.com>
Subject: RE: what else?

Personal Matters / Ex. 6 *But*, I am planning to come Thursday (and I'm here today), possibly Friday afternoon, if you want to have the conversation before then.

-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Thursday, April 11, 2019 4:49 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>

Cc: Jerry Campbell <JCampbell@ramboll.com>
Subject: RE: what else?

Thanks Paul.

How about Monday, April 22, at 10:00?

If you're up for coming to Ramboll, we're still in the old CIIT/Hamner building. Just give Jerry a call on his cell when you get there Personal Matters / Ex. 6 My cell # is below.

With kind regards
Harvey Clewell
PhD, DABT, FATS
Principal Consultant
Ramboll Environment and Health Consulting
Research Triangle Park, NC 27709 USA
hclewell@ramboll.com
919-452-4279

From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Thursday, April 11, 2019 4:23 PM
To: Harvey Clewell <HClewell@ramboll.com>
Cc: Jerry Campbell <JCampbell@ramboll.com>
Subject: RE: what else?

I'm here, open schedule those days. April 22 gives you one more day. I wasn't aware of that deadline!

My practice has been to keep other EPA folk in the loop, but I think this is OK for a discussion of technical details.

Where is Ramboll?

I have to leave now, may be off line until Tuesday. But just pick a time either of those days, morning preferred!

-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Thursday, April 11, 2019 4:14 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Jerry Campbell <JCampbell@ramboll.com>
Subject: what else?

Hi Paul

Would it be possible for us to schedule a time on April 22/23 for an informal discussion of the results of the kg study and Jerry's re-estimation of the metabolism parameters? I know you're busy this week and next, but I've been told that the EPA is requiring us to provide the final version of our PBPK model report by May 1, and I would not like to have to submit it without being able to get your thoughts on some of the technical issues first. I was envisioning it just being me and Jerry. We could meet at EPA, Ramboll, or the Starbucks on 54 (that's where John Wambaugh and I usually meet).

With kind regards
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Message

From: Schlosser, Paul [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=121CF759D94E4F08AFDE0CEB646E711B-SCHLOSSER, PAUL]
Sent: 6/4/2019 7:36:23 PM
To: Harvey Clewell [HClewell@ramboll.com]
CC: Jerry Campbell [JCampbell@ramboll.com]; Michael Dzierlenga [MDZIERLENGA@ramboll.com]; Robinan Gentry [rgentry@ramboll.com]; manderse[Personal Matters / Ex. 6] Thayer, Kris [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3ce4ae3f107749c6815f243260df98c3-Thayer, Kri]; Vandenberg, John [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=dcae2b98a04540fb8d099f9d4dead690-Vandenberg, John]; Bahadori, Tina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7da7967dcafb4c5bbc39c666fee31ec3-Bahadori, Tina]; Morozov, Viktor [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=03cc9abb639c453fabc2bbb3e4617228-Morozov, Viktor]; Davis, Allen [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=a8ecee8c29c54092b969e9547ea72596-Davis, Allen]; Sasso, Alan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8cb867519abc4dcea88149d12ef3e8e9-Sasso, Alan]; Kapraun, Dustin [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3a53c151b92a472fbfb295ed5df982a7-Kapraun, Du]; Sonja Sax [SSax@ramboll.com]; Ken Mundt [kenneth.mundt@cardno.com]; Miyoung Yoon[Personal Matters / Ex. 6]
Subject: RE: chloroprene -- Bayesian analysis

Harvey,

I was looking for a way that the Kg data and the metabolic data might both be consistent, but given the difference in Kg that you got when fitting it, I believe they are not. The question is, why not?

The discussion from Gillette is in regards to hepatocytes, where the concentration of protein will be much higher than a dilute incubation mixture. Another option for testing the in vitro system would be to leave out the NAD(P)H needed for CYP metabolism, yes? But even with heat inactivated proteins one could see if the presence makes any difference at all. Part of Kg is the diffusional resistance in the air above the medium (the "g" in "Kg" is for "gas"), which won't be affected at all by the microsomes.

It's really a very simple physical system. To say that the process of gas-liquid transport is experimentally intractable seems real disparagement of those who work on such things in the lab on a regular basis. I suggested the original reference from Filser's lab in part to make the point that this is not just my pet idea. This question indicates the type of expertise we might need to include on the peer review panel, who will be ultimate judge. Is it possible that the presence of 0.5 mg/ml microsomes causes the mass-transfer rate to increase over 10x above what was measured in the supporting experiments?

A relatively simple test would be to repeat the active mouse liver metabolism experiments under the same conditions, to see if one still saw a rate of uptake from the headspace faster than the Kg experiments seem to support. I'm *not* saying that needs to be done now, but given that the people/lab were the Kg experiments were conducted are different from the one where the metabolic experiments were conducted, this would be a useful test of your hypothesis that somehow presence of the microsomes increases mass transfer that much. It's generally not a good idea to mix control data from one lab, set of experiments, with another, right?

I do not question that Matt Himmelstein wasn't performing his experiments in the best way that he knew. But an even longer-time standard in enzyme kinetics is to provide data showing that the rate is proportional to enzyme (or microsomal) content. Such data would prove that it is enzyme activity which is rate limiting, not something else like mass transport. I looked for such data in the Haskell report, didn't find them. The methods section says that the microsome level was evaluated before settling on 0.5 mg/ml, so the data may have been collected but not reported. But it leaves a doubt.

Previously we discussed whether differences in Km between the liver and lung (and kidney) were real. If you can fit these data using an average Km from across multiple chemicals, then the value cannot be known with great precision. I'm not saying that the value isn't approximately correct, but that this is a confounding factor which contributes to the uncertainty. Data from other chemicals could be used to set an informative, narrower prior on the Km, rather than an uninformative one, if we are sure of those data. But maybe the data from those other chemicals are subject to the same issue? I thought that, if not testing linearity with protein content, measuring Kg was a standard for such experiments. It seems not.

In the end, maybe that doesn't matter because in vivo the metabolism is flow-limited. I do want to keep that in mind.

-Paul

From: Harvey Clewell <HClewell@ramboll.com>
Sent: Tuesday, June 04, 2019 2:12 PM
To: Schlosser, Paul <Schlosser.Paul@epa.gov>
Cc: Jerry Campbell <JCampbell@ramboll.com>; Michael Dzierlenga <MDZIERLENGA@ramboll.com>; Robinan Gentry <rgentry@ramboll.com>; manderser[Personal Matters / Ex. 6]; Thayer, Kris <thayer.kris@epa.gov>; Vandenberg, John <Vandenberg.John@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Morozov, Viktor <Morozov.Viktor@epa.gov>; Davis, Allen <Davis.Allen@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Kapraun, Dustin <Kapraun.Dustin@epa.gov>; Sonja Sax <SSax@ramboll.com>; Ken Mundt <kenneth.mundt@cardno.com>; Miyoung Yoon
[Personal Matters / Ex. 6]
Subject: RE: chloroprene -- Bayesian analysis

Hi Paul

I do not agree that the apparent discrepancy between the Kg experiments and the metabolism experiments leads to parameter uncertainty. To break the collinearity between Km and Kg, we have followed your suggestion of fixing Km at a value based on the literature for cyp2e1 substrates (1 uM), and have re-estimated Vmax and Kg in the male mouse liver, which shows the highest rates of metabolism. The resulting value of Kg represents the maximum limitation on transport in the in vitro studies that is consistent with the data. It does not demonstrate, however, that there was any significant transport limitation in those studies.

Personally, I have complete confidence in the metabolism data collected by Matt Himmelstein and in the approach he used for its analysis. I see no evidence to support an assumption of transport limitation in his studies. The constants derived in the published analysis are consistent with a large body of work on *in vivo* estimation of kinetic constants for clearance of well-metabolized vapors (i.e., with relatively low Km values). Moreover, assuming that there was a significant limitation on transport in these studies results in Km values that are implausibly low, which in turn results in lower risk estimate compared to use of the published values.

The discussion below of the role of plasma proteins on metabolism is from James Gillette's 1973 paper in the Annals of the NY Academy of Science. He suggested that binding proteins in the plasma can accelerate metabolism by acting as carriers of a drug to the vicinity of the hepatocytes. I believe that microsomal proteins can play a similar role in the *in vitro* studies: in the presence of mixing, non-specific binding of a lipophilic compound to proteins can serve to overcome the transport limitation associated with penetrating the aqueous media. In other words, since chloroprene is lipophilic, diffusion through the aqueous media in the *in vitro* assay would normally be rate-limiting, but if the media is well-mixed and contains microsomal proteins, then non-specific binding of chloroprene to the microsomes could greatly enhance its availability for metabolism.

Unfortunately, investigating this effect in the *in vitro* system would not be at all straightforward, because the microsomal proteins serve both as the site of metabolism and as a source of non-specific binding that competes with the surface of the vial. In fact, I do not believe it is possible to experimentally determine a Kg that would be appropriate in any microsomal metabolism study, because of the dual role that the microsomes play (metabolism and non-specific binding). Denaturing the microsomal proteins in order to eliminate metabolism would also alter their tertiary binding structure.

it is impossible to predict when the dissociation of the drug-protein complex is rate limiting from the association constants alone. It seems probable, however, that the rate of dissociation of the drug-protein complex seldom becomes rate limiting in the metabolism of drugs.

It seems more probable that diffusion of the free drug from the plasma into hepatocytes may be the rate-limiting step in the metabolism of drugs by highly active enzymes in the liver. When this occurs, the bound form of the drug in plasma remains in equilibrium with its free form as the concentration of unbound drug in plasma declines during the passage of the blood through the hepatic sinusoids. However, it is also possible that drug-binding proteins in the cytoplasm of hepatocytes hasten metabolism by maintaining the concentration gradient across the membranes of the hepatocytes and by increasing the amount of drug available to the enzymes located toward the central parts of the cells. Indeed, Y and Z proteins [18–21] are thought to act as carriers of sulfobromophthalein and other anions. But, whether they actually act as carriers or as tissue stores of the anions depends on a number of factors that include the association constants and the concentrations of the proteins as well as on the maximum rate and the K_m values of the drug-metabolizing enzymes and the relative diffusivities of the unbound drug and drug-protein complexes. Because of the complexities of these interrelationships, it is frequently difficult to determine when binding sites in hepatocytes serve as transport mechanisms or as storage mechanisms.

To determine whether plasma proteins act as transport carriers, it is necessary to calculate the apparent clearance on the basis of the concentration of unbound drug in plasma and to compare these apparent clearance values (rate of elimination/concentration of unbound drug) with hepatic blood flow rate. If the apparent clearance exceeds the hepatic blood flow when the drug is known to be eliminated solely by the liver, it may be concluded that the plasma proteins or other components in blood act as transport carriers. When it is not known whether a drug is eliminated solely by the liver, it would be useful to determine whether the clearance value exceeds the cardiac output, because the true clearance can never exceed the cardiac output.

With kind regards

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